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URINARY DEHYDROISOANDROSTERONE IN HYPERADRENOCORTICISM: INFLUENCE OF CORTISONE, HYDROCORTISONE AND ACTH*

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INTRODUCTION

THIS paper deals with a comparison of studies made on 4 patients with adrenocortical tumors, 6 patients with congenital adrenal hyperplasia and 1 patient with postnatal adrenal hyperplasia. The observations include a) spectrophotometric curves of a colorimetric reaction for dehydroisoandrosterone performed on urinary extracts (1), b) measurement of the total amounts of 17-ketosteroids and dehydroisoandrosterone-like steroids (DHIA steroids) excreted, and c) effects of cortisone and ACTH on the excretion of the various groups of urinary steroids which were measured.

In 1936 Callow isolated dehydroisoandrosterone as the prominent uri-

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nary steroid in a case of virilizing adrenocortical tumor (2). Talbot, Butler and MacLachlan in 1940 found by digitonin precipitation that urinary excretion of the 3-beta-hydroxy-17-ketosteroids was abnormally increased in children with virilizing adrenocortical tumor (3). These studies have since been repeatedly confirmed (4–7). There appeared to be no simple, colorimetric way of differentiating between the 3-alpha-hydroxy and 3-beta-hydroxy urinary 17-ketosteroids until Dirscherl and Zilliken discovered that pure dehydroisoandrosterone gave a blue color on treatment with concentrated sulfuric acid and dilution with water (8). Subsequently several workers have adapted this type of reaction for use with urine extracts, since it clearly distinguishes dehydroisoandrosterone from androsterone (1, 9, 10). Allen and colleagues have prepared spectrophotometric curves from patients with virilizing adrenocortical tumor, adrenocortical hyperplasia and Cushing's syndrome, using a variation of this colorimetric procedure (1). These workers found that there were striking differences between the spectrophotometric curves of patients with virilizing adrenocortical tumor and those with adrenocortical hyperplasia or Cushing's syndrome.

The present study confirms these findings, and extends them to include observations on such patients treated with cortisone, hydrocortisone (compound F; 17-hydroxycorticosterone) and ACTH. The fact that cortisone in relatively small doses suppresses excessive androgen production and 17-ketosteroid excretion in congenital adrenal hyperplasia has been reported in a series of papers from this and other clinics (11–19). Venning has reported that in a patient with adrenal adenoma, cortisone failed to reduce the output of urinary 17-ketosteroids (20).

METHODS

Twenty-four-hour urine collections were preserved with 15 ml. of 25 per cent acetic acid. It was found necessary, particularly in the cases of virilizing tumor, to refrigerate the specimens during collection, and to perform the chemical determinations promptly. The following methods were used:

(a) *The 17-ketosteroid determinations* were made as follows: An aliquot of 100 ml. of urine from a 24-hour specimen plus 10 ml. of concentrated HCl was hydrolyzed by boiling for fifteen minutes. This material was cooled and extracted once with 40 ml. of carbon tetrachloride and twice with 30 ml. of carbon tetrachloride. The carbon tetrachloride was evaporated to dryness in vacuo at 75° C. This residue was dissolved in ethyl ether and washed with 2.5 N sodium hydroxide. The sodium hydroxide aqueous extract was discarded and the ether evaporated to dryness on a steam bath. The residue was dissolved in 2 ml. of redistilled absolute ethyl alcohol. This residue was used for the Zimmermann reaction for 17-ketosteroids (meta-dinitrobenzene in ethanolic KOH), using androsterone as a standard.

(b) *The DHIA reaction* was performed by a slight modification of the method of Allen and colleagues (1). An aliquot of 0.2 ml. of the previously mentioned alcoholic

residue was evaporated to dryness over a steam bath. In some urines with very high ketosteroid values a smaller aliquot was used. Three ml. of an ethanol sulfuric acid solution (1 volume of 95 per cent ethanol: 4 volumes of concentrated sulfuric acid) was added and the flask was kept in a water bath at 55° C. for twelve minutes. The flask was cooled for one minute in ice water and 4 ml. of 95 per cent ethanol was added with swirling. The flask was cooled again in water. The colors developed immediately, as previously described. Quantitative measurement of 24-hour excretion of DHIA compounds was made by constructing a calibration curve with pure dehydroisoandrosterone acetate as a standard (concentrations were calculated as free dehydroisoandrosterone). Measurements of optical density were made with a Beckman spectrophotometer, model DU, using cuvettes with a 1-cm. light path. The ultraviolet-sensitive phototube was used for measurements below 625 mμ. Good linearity was obtained between 12.5 and 75.0 micrograms of dehydroisoandrosterone per 7 ml. of final solution. In order to correct for interfering colors, the correction equation as derived by Allen (21) was applied: Calculated Optical Density at 600 mμ or "CDA600" = Optical Density at 600 mμ − $\left(\frac{\text{Optical Density at 560 m}\mu + \text{Optical Density at 640 m}\mu}{2}\right)$. The working calibration curve was then made up with CDA600 values on the ordinate, and micrograms of dehydroisoandrosterone per 7 ml. of solution on the abscissa. Optical density measurements were made at the three wave lengths, 560 mμ, 600 mμ and 640 mμ. CDA600 was calculated by substituting in the foregoing equation, and the result in micrograms of dehydroisoandrosterone per 7 ml. of final solution was read off the calibration curve. The result was then calculated on the basis of 24-hour urine volume, and expressed as mg. of dehydroisoandrosterone per twenty-four hours.

(c) *Spectrophotometric curves* of the foregoing reaction between 300 mμ and 700 mμ were obtained, using the technique of Allen and colleagues (1). Because it was not possible to use exactly the same amount of steroid in each preparation, the position of each curve on the optical density axis varied. Hence only the configuration of the curve, not the position on the optical density axis, is of importance.

(d) *Relatively water-soluble reducing corticoids* (neutral reducing lipids; "11-oxysteroids") of urine (22) were determined by the phosphomolybdic-acid color reaction on chloroform extracts (23) which had been subjected to benzene-water partition but not treated with Girard's reagent. The upper limit of normal for adults by this method is 1.0 mg. per day.

RESULTS

I. *Data on normal subjects*

Figure 1 shows the configuration of DHIA reaction spectral absorption curves obtained on urine extracts from normal male and female subjects. The two left-hand absorption maxima (at approximately 390 mμ and 490 mμ) are probably due to androsterone and other related steroids (1). The very small peak at 600 mμ in the normal curve occurs at the maximum absorption for pure dehydroisoandrosterone acetate and may represent the small daily excretion of dehydroisoandrosterone by the normal adult. Earlier studies (24) showed that normal adults excrete 0.2 to 0.4 mg. per day of dehydroisoandrosterone, and our controls agree

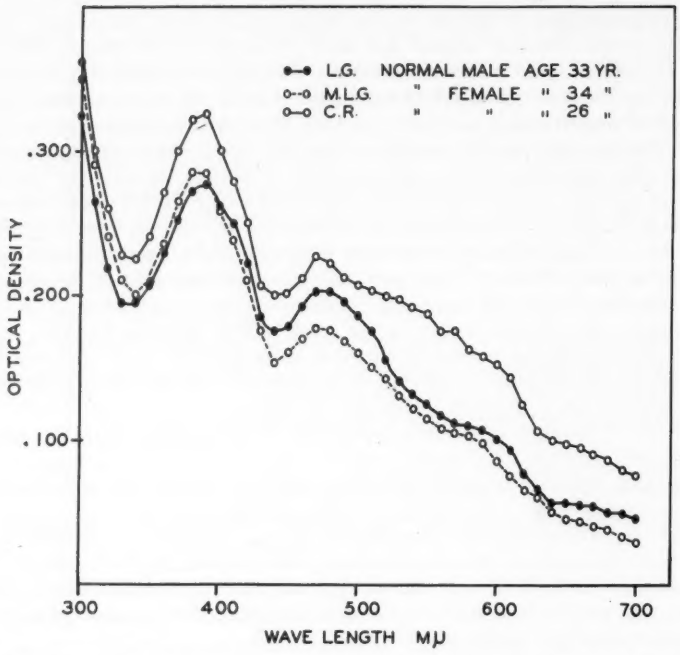

FIG. 1. Spectral absorption curves of the DHIA reaction on urine steroids in 3 normal adult subjects. (*See* Table 2 for amounts of steroid residue used in constructing these and subsequent absorption curves.)

with these figures. However, recent experiments in which the optimal conditions for hydrolysis and extraction may have been more nearly attained, indicate that adults may excrete as much as 1 to 2 mg. per day of dehydroisoandrosterone (25).

II. *Data on patients with adrenocortical tumors*

M.B. (*A-85294*) was a white female who was referred to this clinic at the age of $3\frac{2}{12}$ years because of pubic hair which had appeared at the age of $2\frac{8}{12}$ years. She was 2.5 inches above the average for her height and had a height-age of 4 years. Her bone age was 6 years. The skin showed no acne, and there was no change of voice. Blood pressure was 95/60. No abnormal masses were palpated in the abdomen. The clitoris was only slightly enlarged, measuring 1.7 cm. in length. There was a separate urethra and vaginal orifice in the usual position. No pelvic organ or mass could be felt by rectum.

Urine examination and blood counts gave normal results. No abnormalities were noted in the blood chemical determinations (Table 1). A virilizing adrenocortical tumor was suspected and attempts were made to localize it. Pyelograms did not reveal any displacement of the kidneys or any mass in the adrenal regions. Radiograms showed no metastases in the lungs or bones.

TABLE 1. DATA ON TOTAL BLOOD EOSINOPHILS, HEMATOCRIT AND SERUM CHEMICAL DETERMINATIONS BEFORE AND AFTER ADMINISTRATION OF ACTH TO 2 PATIENTS WITH VIRILIZING ADRENOCORTICAL TUMORS. URINARY STEROID MEASUREMENTS WERE MADE ON SPECIMENS COLLECTED THE DAY BEFORE AND THE DAY OF ACTH THERAPY

Patient	Therapy	Blood		Serum					Urine			
		Eosinophils per cu. mm.	Hematocrit, %	Total protein, Gm. %	Na mEq./L.	K mEq./L.	Cl mEq./L.	CO_2 mEq./L.	Vol. cc./d.	17-KS mg./d.	DHIA mg./d.	11-OS mg./d.
M. B.	Control	140	38.5	—	148	—	114	25	630	40	20	0.45
	ACTH, 50 mg. i.v.	0	39	—	137	6.5	105	23	550	50	16	0.49
H. B.	Control	462	—	7.3	147	4.7	104	29	900	194	162	1.49
	ACTH, 50 mg. i.v.	311	—	5.9	127	4.1	97	23	945	231	165	3.79

The patient was given ether anesthesia and a polyethylene catheter inserted into the right femoral vein and another into the left femoral artery.[1] These were pushed up into the vena cava and aorta, respectively, to the level of the renal veins and arteries. One hundred ml. of blood was removed from vena cava and aorta for lyophilization and was sent to the Worcester Institute for Experimental Biology, Shrewsbury, Massachusetts, for steroid analyses. After injection of Diodrast into the aorta there was seen some increased vascularity over an area just above the right kidney in the region of the right adrenal gland. After completion of studies on the urinary steroids to be described, a well circumscribed right adrenal tumor, 6 cm. in diameter, was removed via a transthoracic approach. Postoperatively the patient was given no hormonal therapy, and made an uneventful recovery. The tumor was flown to the Worcester Institute for Experimental Biology, where perfusion studies were carried out.

Studies of urinary steroids. The preoperative spectrophotometric curve obtained on the urinary steroids of this patient showed a very pronounced absorption at 600 mμ. Postoperatively this absorption peak essentially disappeared (Fig. 2). During a 6-day control period the output of DHIA steroids varied between 20 and 30 mg. per day, with an average of 25 mg. per day; the 17-ketosteroids varied between 29 and 58 mg. per day, with an average of 41 mg. per day; and the reducing corticoids were between 0.45 and 0.71 mg., with an average of 0.59 mg. per day (Fig. 3). At the end of the control period 50 mg. of ACTH was given intravenously over twelve hours. Table 1 shows no significant change in the excretion of any of the urinary steroids as a result of ACTH treatment. Beginning on the eleventh day of study, cortisone was administered in the dosage of 100 mg. per day orally for five days. The urinary excretion of DHIA steroids,

[1] The authors are indebted to Dr. E. Converse Peirce, Dept. of Surgery, U. S. Public Health Service Hospital, Baltimore, Md., for carrying out the aortic angiogram, and to Dr. Charles Tesar, Dept. of Urology, Johns Hopkins Hospital, for lyophilizing the blood specimens.

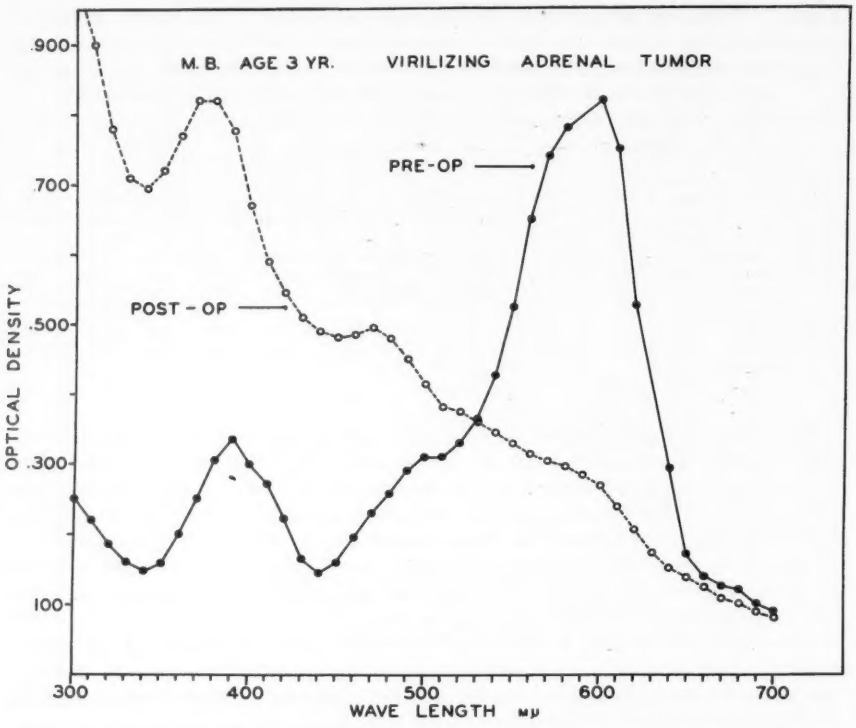

FIG. 2. Preoperative and postoperative spectral absorption curves of the DHIA reaction on urinary steroids of a female child with a virilizing adrenocortical tumor.

17-ketosteroids and reducing corticoids appeared to increase during the period of cortisone therapy, with a tendency for the DHIA steroids and 17-ketosteroids to fall after therapy was discontinued. Postoperatively no measurable DHIA steroids were excreted, and 17-ketosteroids fell to values of 0.3 and 1.1 mg. per day, with an average of 0.8 mg. per day. In the third month after discharge from the hospital, urinary 17-ketosteroids were between 0.5 mg. and 1.6 mg. per day.

H.B. (*A-84056*) was a white male, aged $4\frac{3}{4}$ years, in whom an enlarged penis and pubic hair developed at the age of 3 years. A $6\frac{1}{2}$-year-old sibling was living and normal. There had been no known endocrine disorder in the family. At the age of 3 years enlargement of the penis was noted, and a few months later pubic hair appeared. At the age of $3\frac{5}{12}$ years he was studied at Duke University Hospital, where he was found to have a height-age of 4 years and a bone age between 4 and 6 years. Pyelograms showed the presence of a mass on the left side of the abdomen, depressing the left kidney. The 17-ketosteroids were reported on two different 24-hour urine specimens as: alpha fraction, 87.3 and 143.7 mg.; beta fraction, 73.7 and 143.7 mg. An assay for urinary gonadotropins

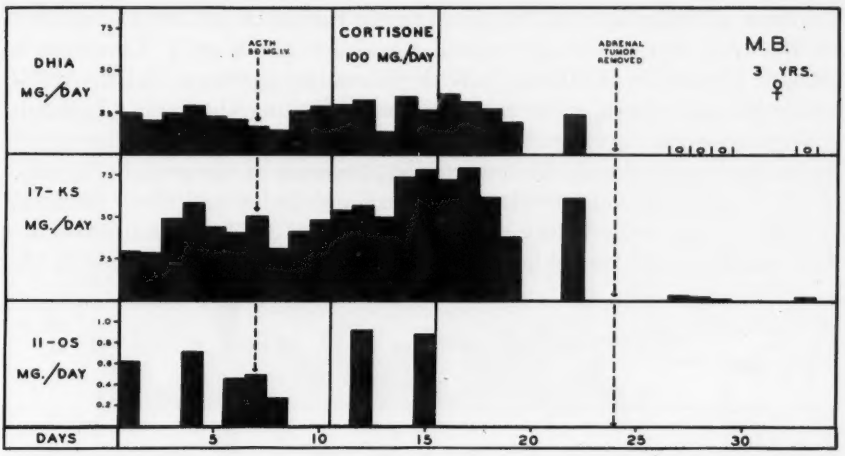

FIG. 3. Effects of ACTH therapy, cortisone therapy and operation on the urinary excretion of DHIA steroids, 17-ketosteroids and reducing corticoids in a female child with virilizing adrenocortical tumor.

gave negative results. Operation was recommended but was refused. The child remained in apparent excellent health but continued to grow and virilize excessively.

When examined at the Harriet Lane Home at the age of $4\frac{3}{4}$ years his height-age was 8 years and his bone age, 12 years. There was no clinical evidence of Cushing's syndrome. There was marked, advanced development of the penis, scrotum and prostate. There was abundant pubic hair and a deep voice. Both testes were in the scrotum and measured 2.7×1.5 cm., thus showing no appreciable enlargement for his age. Blood pressure varied between 104/54 and 112/72. At the level of the umbilicus a rounded mass was felt deep in the left abdomen, which was thought to be a displaced kidney. This was confirmed by intravenous pyelograms. Urine examination and blood count gave normal results, save for a moderately elevated total eosinophil count (Table 1). The serum electrolyte values shown in Table 1 were normal. A glucose tolerance curve was normal, with a fasting value of 96 mg. per cent. X-ray examination of the bones revealed no evidence of metastases. Aortic angiograms were made by the technique previously described and 200 ml. of blood was taken from the aorta and vena cava each, lyophilized and sent to the Worcester Institute for Experimental Biology for steroid analyses. After the completion of studies to be described, the patient was operated on by Drs. W. W. Scott and H. W. Scott and a well-encapsulated tumor, 5 cm. in diameter, was removed through a left transthoracic incision. The tumor was flown to the Worcester Institute for Experimental Biology where it was subjected to perfusion studies with ACTH, cortisone and compound S (17-hydroxydesoxycorticosterone). Other than cortisone preoperatively, no hormonal therapy was used either preoperatively or postoperatively. Recovery was uneventful.

Studies of urinary steroids. Due to the lability (26) of the 3-beta-hydroxy-ketosteroids which made up a large fraction of the total urinary 17-ketosteroids, all urine samples were kept in a ward refrigerator during

the time of collection. Spectrophotometric curves of the DHIA reaction on the urine steroids of this patient are shown in Figure 4. There was a marked absorption at 600 mμ in both the control specimen and the specimen obtained during cortisone therapy, indicating that this treatment did not suppress excretion of the DHIA steroid fraction. The absorption curve made postoperatively showed disappearance of the peak at 600 mμ. Table 1 shows the data obtained from urinary steroid and blood chemical determinations made before and after 50 mg. of ACTH was injected intravenously over a twelve-hour period. There appeared to be a drop in the

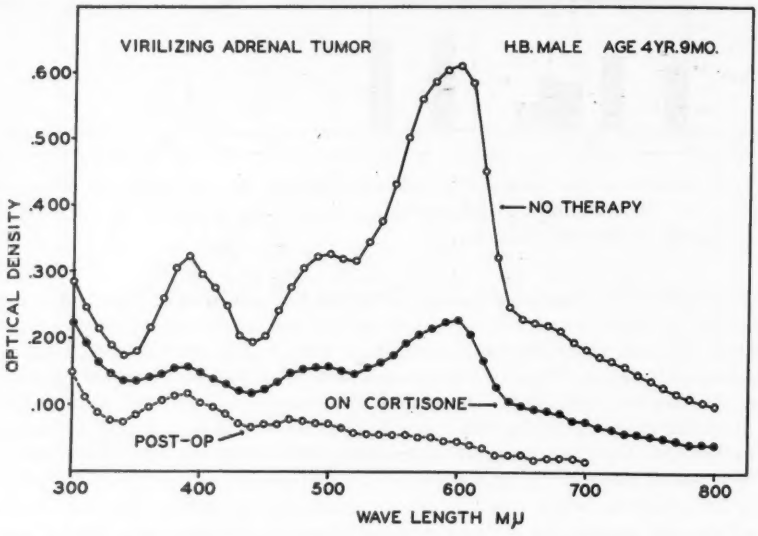

FIG. 4. Spectral absorption curves of the DHIA reaction on urinary steroids of a boy with virilizing adrenocortical tumor. The peak at 600 mμ persisted while he was receiving cortisone, but disappeared postoperatively.

levels of serum sodium, chloride and CO_2 combining power, with no change in the urinary excretion of DHIA steroids or 17-ketosteroids. There was no apparent increase of urinary reducing corticoids during ACTH therapy.

Figure 5 shows the urinary excretion studies of DHIA steroids, 17-ketosteroids and reducing corticoids made on this patient. On the third day after the aortic angiogram there was a sudden increase of both 17-ketosteroids and DHIA steroids to values of 504 mg. and 405 mg., respectively, which were the highest steroids excretions observed in this patient. Although cortisone in a dosage of 100 mg. was administered orally on this one day, it is unlikely that cortisone caused this sudden rise of urinary steroids, since later treatment with cortisone provoked no such response.

Cortisone acetate was administered orally, 100 mg. per day, for a period of eight days (Fig. 5). There was a temporary decrease of excretion of DHIA steroids and 17-ketosteroids on the second and third days of therapy, and at the same time there was a rise of urinary reducing corticoids. There was also a concomitant water diuresis, with urine volume increasing from a daily average of 1,080 ml. for the control period to a maximum of 2,225 ml. for the second day on cortisone therapy.

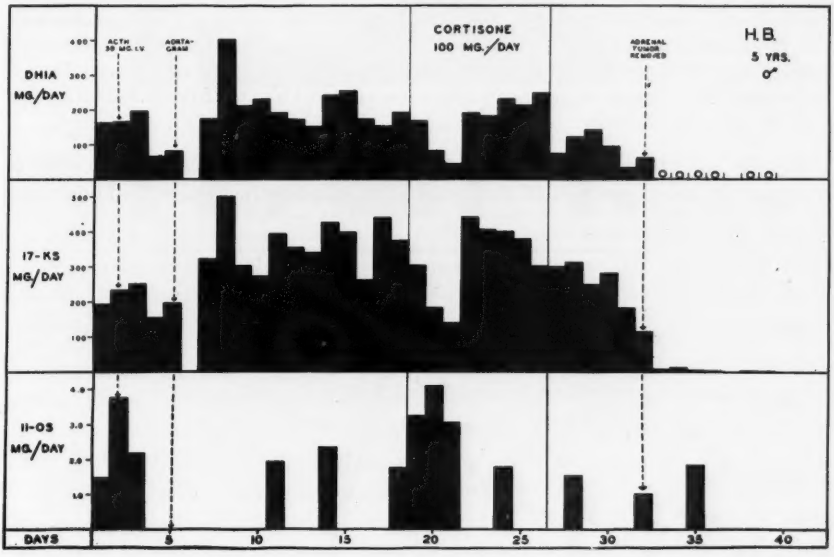

FIG. 5. Effects of ACTH therapy, cortisone therapy and operation on the urinary excretion of DHIA steroids, 17-ketosteroids and reducing corticoids in a boy with virilizing adrenocortical tumor.

Postoperatively DHIA steroid excretion fell to levels too low to assay, and 17-ketosteroid excretion varied between 5.2 and 1.5 mg. per day.

E.H. (*J.H.H. 388102*) was a white female, aged 35 years, who was referred to Dr. G. E. Seegar-Jones because of repeated miscarriages. The patient's growth and development had been normal. She first menstruated at 12 years of age, having periods at 28-day intervals, lasting five days. At the age of 21 years excessive growth of hair appeared on her arms, legs, face and abdomen, increasing progressively for five years. In the thirteen years before admission she had had 11 miscarriages, all between the second and fourth months of gestation. Four years prior to this entry she was studied at the Women's Clinic of the Johns Hopkins Hospital and found to have a bicornuate uterus. Her last miscarriage was at five months and her last menstrual period two weeks before admission.

The patient was of average build with an attractive feminine face from which coarse dark hair had been removed by repeated electrolysis. Her musculature was not exces-

sively developed. There was no obesity, rounding of the face, plethora or body striae. There was coarse black hair over the abdomen, chest, pubis, perineum and thighs. The breasts were of average size and of normal glandular consistency. The labia minora and vulva were well developed. The clitoris was only slightly hypertrophied, measuring 2.5 cm. in length. Blood pressure varied between 108/65 and 130/75. Urine examination, blood count, a glucose tolerance test and the determination of serum sodium and potassium gave normal results. X-ray examination of bones and lungs showed no metastases.

Because of the findings on urinary steroids to be described, an adrenocortical tumor was suspected. Laparotomy was performed by Dr. Howard Jones through a lower midline incision. A plastic repair of the bicornuate uterus was made, and the operator was able to palpate a tumor in the left adrenal region. Following this operation studies were made of the effects of cortisone on the urinary steroids over a period of six days. Then a well-encapsulated tumor weighing 120 grams was removed by Dr. Howard Jones through a left transthoracic incision.

Studies of urinary steroids. Spectrophotometric curves of the DHIA reaction on urine steroids of this patient are shown in Figure 6. There was

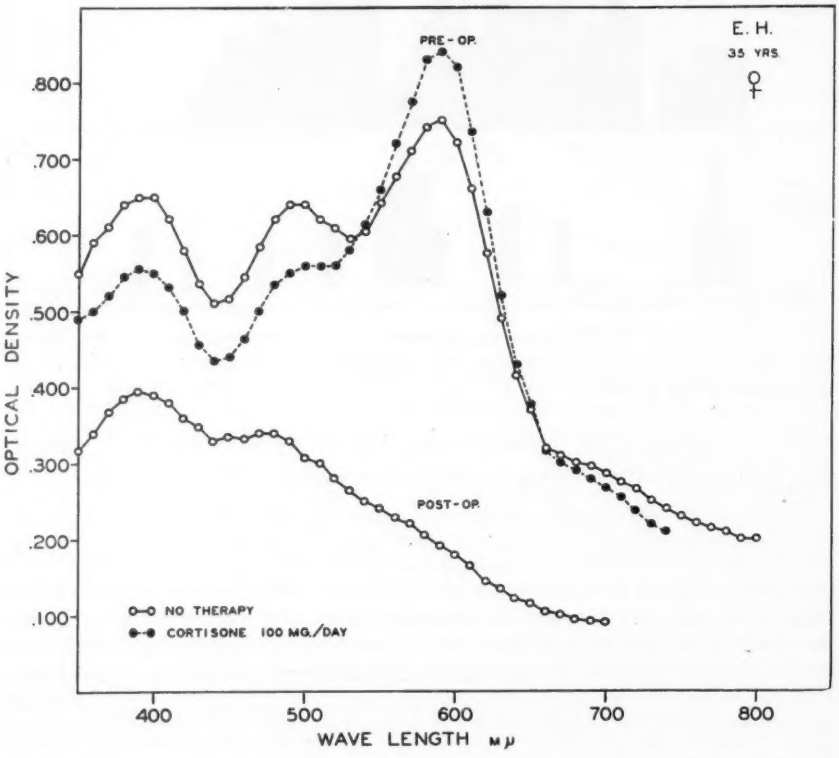

FIG. 6. Spectral absorption curves of the DHIA reaction on urinary steroids of a woman with hirsutizing adrenocortical tumor. The relative height of the peak at 600 mμ increased on cortisone therapy, whereas it entirely disappeared after operation.

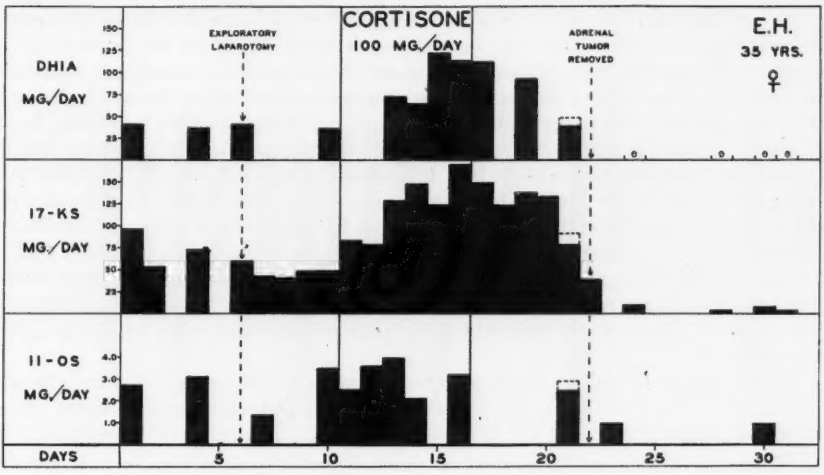

FIG. 7. Effects of cortisone therapy and operation on the urinary excretion of DHIA steroids, 17-ketosteroids and reducing corticoids in a woman with hirsutizing adrenocortical tumor.

a very marked absorption at 600 mμ, similar to that in the 2 previous cases. When cortisone was administered, the peak at 600 mμ became higher in relation to the peaks at 390 mμ and 490 mμ. Postoperatively the absorption peak at 600 mμ disappeared.

Figure 7 shows the studies made on the quantitative output of steroids by this patient. During the five-day control period, two determinations of DHIA steroids were 40 mg. and 37 mg. per day, respectively; 17-ketosteroids ranged from 45 mg. to 96 mg. per day, with an average of 74 mg. per day; and two determinations of reducing corticoids were 2.75 mg. and 3.1 mg. per day, respectively. During a six-day period of treatment with 100 mg. of cortisone acetate per day orally there was a rise in the excretion of DHIA steroids to a maximum value of 123.6 mg. per day, with an average value for the period of 94 mg. per day. The 17-ketosteroids also rose, with a maximum value of 170 mg. per day, and an average for the period of 142 mg. per day. Cortisone therapy did not appear to influence the excretion of urinary reducing corticoids. After cortisone was discontinued there seemed to be a diminution in the urinary excretion of DHIA steroids and 17-ketosteroids.

By the third day after operation DHIA steroids fell to values too low to measure quantitatively by the method used. The urinary 17-ketosteroid excretion fell to a level between 9.9 mg. and 3.6 mg. per day and reducing corticoid excretion fell to values of 0.98 and 0.99 mg. per day. Monthly determinations of urinary 17-ketosteroids made after discharge from the hospital showed values of 6.0 mg., 6.6 mg. and 3 mg. per day.

H.G. (*J.H.H. 561656*) was a white female, aged 42 years, who noted the development of a left supraclavicular lump eight months before entry. The lump was excised and a histologic diagnosis of lymphosarcoma was made at that time, but subsequent examination suggested that it was a metastasis from an adrenocortical tumor. Six months before entry the patient's face became more rounded and moderate hirsutism developed. Subcutaneous bruises became prominent. Her menses stopped entirely, her abdomen began to swell, and there was edema of the legs. Intravenous pyelography showed displacement of the left kidney. X-ray therapy was given to this region. The patient was admitted to the Johns Hopkins Hospital for diagnostic studies by Drs. Grant Ward and John Eager Howard. She did not appear acutely ill, and presented a characteristic picture of Cushing's syndrome, with a round, full face, acne, ecchymoses, plethora and moderate facial hirsutism. Blood pressure was 228/110. There was a firm mass in the left supraclavicular area, 2 cm. in diameter. An indefinite mass in the left abdomen could be felt by some observers.

In keeping with Cushing's syndrome were: eosinopenia, glycosuria, a diabetic glucose tolerance curve, hypochloremic alkalosis and diffuse osteoporosis of the lumbosacral spine and pelvis. Values for blood NPN, and serum sodium and potassium were not abnormal. After the completion of studies to be described the patient returned home. Although there was no objective evidence of benefit from cortisone therapy, the patient experienced such psychologic improvement while receiving it that the drug was continued.

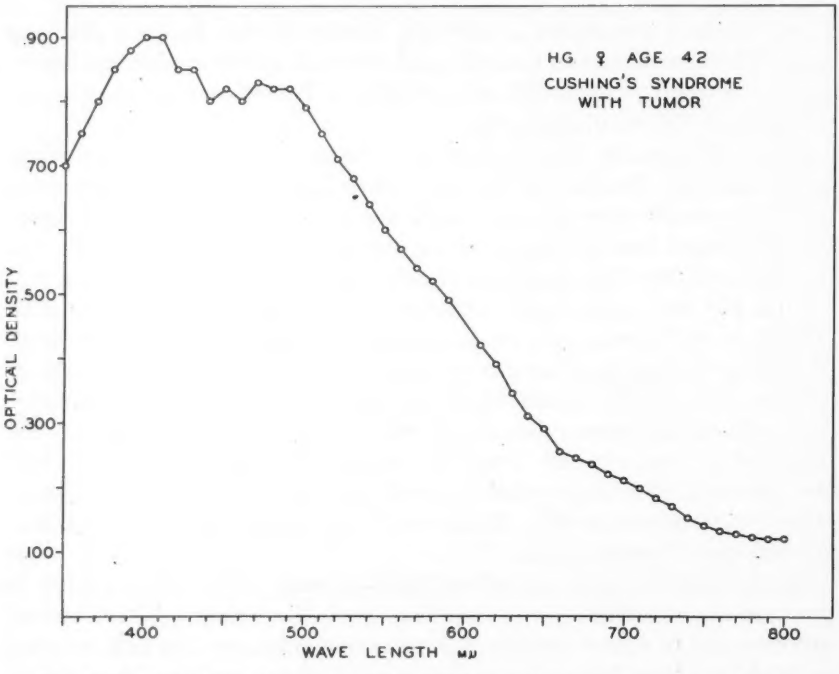

FIG. 8. Spectral absorption curve of the DHIA reaction on urinary steroids of a woman with adrenocortical tumor (Cushing's syndrome type).

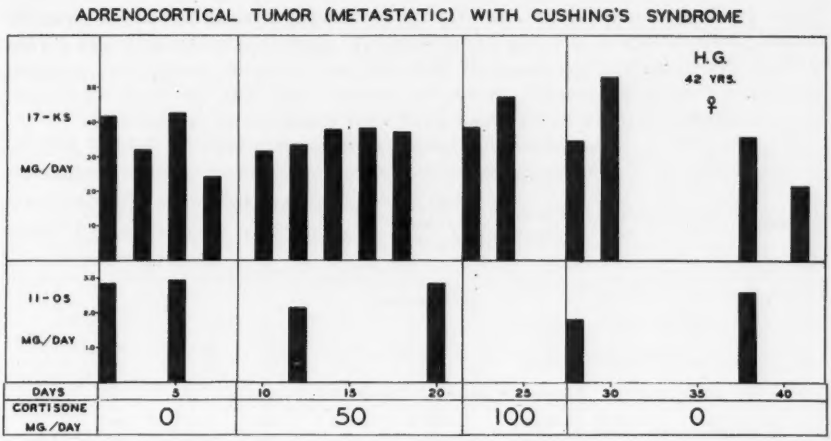

FIG. 9. Lack of effect of cortisone therapy on the urinary excretion of 17-ketosteroids and reducing corticoids in a woman with adrenocortical tumor of the Cushing's syndrome type.

Approximately one year from the date of the original symptoms the patient died at home. Autopsy revealed a purulent peritonitis. There was an oval mass above the right kidney which weighed about 250 grams and was about 15 cm. in diameter. A mass one-third this size was above the left kidney.

Studies of urinary steroids. The spectrophotometric curve made on the urinary steroids of this patient did not show an abnormal absorption peak at 600 mμ (Fig. 8). The excretion of DHIA steroids averaged 1.5 mg. per day. During the eight-day control period prior to cortisone therapy urinary 17-ketosteroids varied between 24 mg. and 42 mg. per day, with an average of 35 mg. per day; and reducing corticoids were 2.8 mg. and 2.9 mg. per day (Fig. 9). Cortisone acetate, 50 mg. per day, was given intramuscularly for thirteen days, then for a period of six days the dose was raised to 100 mg. There was no appreciable change in 17-ketosteroid excretion, which varied between 32 mg. and 47 mg., with an average of 38 mg. per day. Reducing corticoids did not change, with excretions of 2.1 mg. and 2.9 mg. per day.

III. *Data on congenital and postnatal adrenal hyperplasia*

Studies were carried out on 7 patients with adrenocortical virilism who did not have neoplasms or Cushing's syndrome. Of these, 4 were females with pseudohermaphrodism, 2 were males with macrogenitosomia praecox and one was a female of 22 years who became hirsute and virile during adolescence. None of the patients had shown clinical manifestations of sodium loss. One of the female pseudohermaphrodites and the patient with acquired virilism had hypertension, which decreased under cortisone

TABLE 2. COMPARISON OF URINARY STEROID DATA ON 3 NORMAL ADULTS, 4 CASES OF ADRENOCORTICAL TUMOR, 6 CASES OF CONGENITAL ADRENAL HYPERPLASIA AND 1 CASE OF POSTNATAL ADRENAL HYPERPLASIA. NONE OF THE CASES OF CONGENITAL ADRENAL HYPERPLASIA SHOWED EVIDENCE OF EXCESS SODIUM LOSS. THE SPECTROPHOTOMETRIC CURVES IN FIGURES 1–11 WERE MADE FROM ALIQUOTS OF THE URINARY 17-KETOSTEROID RESIDUE AS HERE INDICATED

Subject	Sex	Age, yrs.	Therapy, mg./day	Total 17-KS, mg./day	Total DHIA, mg./day	Urine vol. ml./day	Aliquot urine used, ml.	Aliquot 17-KS used, μgm.	Figure
			Normal adults						
I. G.	M	33	None	16.3	<.5	1,670	10	98	1
M. L. G.	F	34	None	5.0	<.5	1,230	10	40	1
C. R.	F	26	None	4.4	<.5	750	10	58	1
			Patients with adrenocortical tumor (virilizing type)						
M. B.	F	3 2/12	None	37.2	30.6	430	1.0	88	2
			Postop.	0.9	<.5	780	50.0	58	2
H. B.	M	4¾	None	378.0	191.0	910	0.25	103	4
			Cortisone, 100 orally	409.0	185.5	1,550	0.25	66	4
			Postop.	2.9	<.5	730	5.0	19	4
			Patient with adrenocortical tumor (hirsutizing type)						
E. H.	F	35	None	73.7	37.2	1,030	1.25	90	6
			Cortisone, 100 orally	123.6	123.6	2,060	1.25	75	6
			Postop.	7.4	<.5	1,470	10.0	50	6
			Patient with adrenocortical tumor (Cushing's syndrome type)						
H. G.	F	42	None	41.9	1.5	2,700	10.0	155	8
			Patients with congenital adrenal hyperplasia						
B. H.	M	3	None	6.8	1.3	530	2.5	20	—
			Cortisone, 50 i.m.	1.4	<.5	1,000	10.0	14	—
T. T.	M	6	None	36.0	<.5	500	5.0	360	—
			Cortisone, 100 i.m.	3.7	<.5	660	10.0	56	—
P. R.	F	15½	None	43.0	2.6	765	5.0	281	—
			Cortisone, 50 orally	26.3	1.1	910	10.0	289	—
M. N.	F	10	None	22.8	1.8	1,440	5.0	79	—
			Cpd. F, 25 i.m.	6.9	<.5	920	10.0	75	—
W. D.	F	10	None	48.0	3.2	815	2.5	143	—
			ACTH, 50 i.v.	68.0	12.3	2,070	1.25	41	—
M. C. S.	F	16½	None	70.0	8.9	740	2.5	236	10
			Cortisone, 50 i.m.	6.8	<.5	780	10.0	87	10
			Patient with postnatal adrenal hyperplasia						
J. J.	F	22	None	33.0	6.0	2,010	10.0	160	11
			Cortisone, 50 i.m.	7.9	<.5	1,900	10.0	41	11

therapy. Most of these patients have been described in previous papers in which the effects of cortisone were discussed (11–19). The essential data are summarized in Table 2.

Spectrophotometric curves. Five of the patients showed spectrophotometric curves with configurations similar to the normal (Fig. 1). Two patients (M.C.S. and J.J.) had spectrophotometric curves with distinct peaks at 600 mμ (Figs. 10 and 11). It is of interest that both of these patients had hirsutism, pigmentation and hypertension.

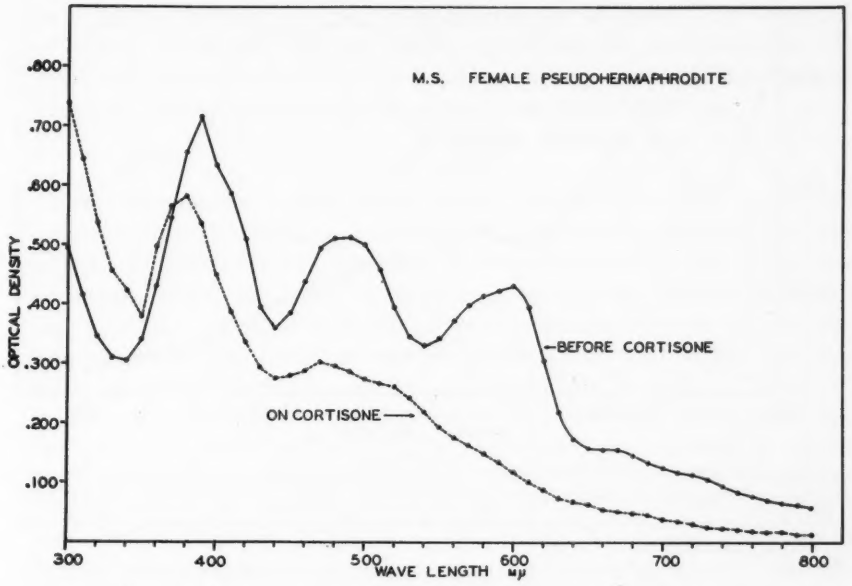

FIG. 10. Spectral absorption curves of the DHIA reaction on urinary steroids in a female pseudohermaphrodite with hypertension. With cortisone treatment the peak at 600 mμ disappeared.

Amounts of DHIA steroids excreted. In most instances the amount of DHIA steroids was too low to be calculated accurately from the calibration curve, and in untreated cases varied from less than 0.5 mg. per day to 3.2 mg. per day. In the 2 patients (M.C.S. and J.J.) having peaks at 600 mμ, it was calculated that 8.9 mg. and 6.0 mg. per day respectively were excreted, which is unquestionably above the normal range. It is noteworthy that in 1944 Frame, using the digitonin method, found that M.C.S. at the age of 9 years excreted 4.4 mg. per day of 3-beta-hydroxy-17-ketosteroids, which was 8.5 per cent of the total 17-ketosteroid output (6).

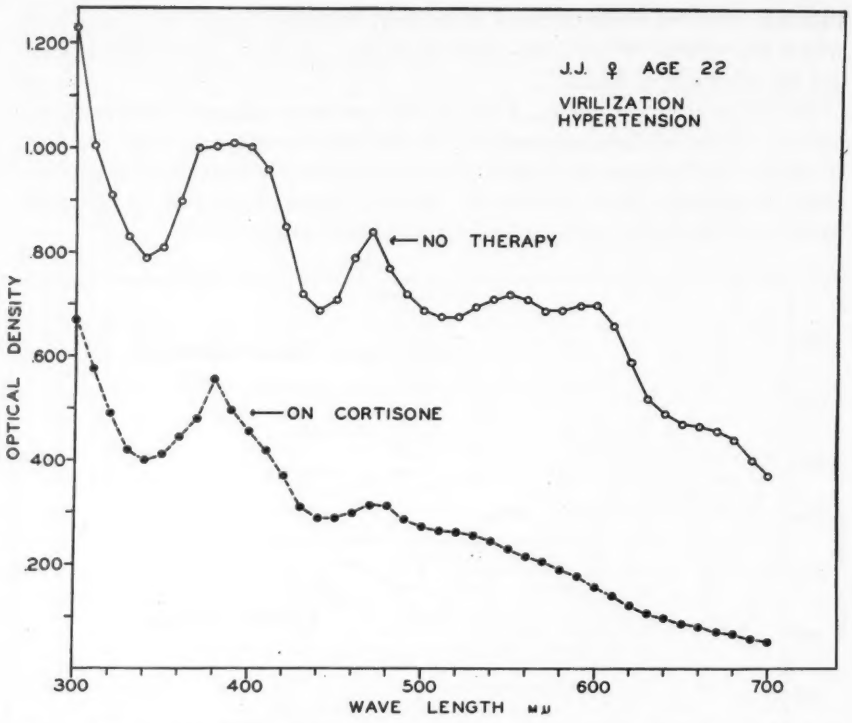

FIG. 11. Spectral absorption curves of the DHIA reaction on urinary steroids in a woman with postnatal virilization and hypertension. When cortisone was administered there was narrowing of the peak at 390 mμ and nearly complete obliteration of the peaks at 550 mμ and 600 mμ.

At the age of $16\frac{1}{4}$ years the output of 8.9 mg. DHIA steroid per day accounted for 12.7 per cent of her total 17-ketosteroids.

Effect of cortisone and hydrocortisone (compound F) on the spectrophotometric curve. In 4 cases (B.H., T.T., P.R. and M.C.S.) the spectrophotometric curve was studied while the patients were receiving cortisone, and the total urinary output of 17-ketosteroid was suppressed, as shown in Table 2. In the patients who exhibited curves of normal configuration before treatment there were no very marked changes in configuration. Patient B.H. showed suppression of his slight elevation at 600 mμ. The differences in position of these curves on the optical density axis are due to the use of varying aliquots of steroid residue for the DHIA reaction. In the 2 patients (M.C.S. and J.J.) who had abnormal peaks at 600 mμ before treatment, the peaks disappeared while cortisone was being given (Figs.

10 and 11). It is significant that the hypertension was decreased under this treatment (19).

In 1 patient (M.N.) the previously normal curve evidenced some flattening of the elevation at 600 mμ as a result of intramuscular administration of 25 mg. of hydrocortisone (compound F) per day.

Effect of ACTH on the spectrophotometric curve. Patient W.D. was given a constant intravenous infusion of 50 mg. of ACTH over a twelve-hour period. During this day the excretion of urinary 17-ketosteroids increased to 68 mg. from an average output of 47 mg., and the DHIA steroids rose from 3.2 mg. to 12.3 mg. This disproportionate increase in the excretion of DHIA steroids as compared with 17-ketosteroids is reflected in the spectrophotometric curve. The elevation at 600 mμ in the ACTH curve is increased in height relative to the peaks at 400 mμ and 490 mμ.

DISCUSSION

Spectrophotometric curves of the DHIA reaction. The data herein described suggest three principal patterns of spectrophotometric curves of the DHIA reaction on urine extracts in hyperadrenocorticism: a) curves similar to the normal, seen in most cases of congenital adrenal hyperplasia and Cushing's syndrome; b) curves with a moderate peak at 600 mμ, which is obliterated by cortisone therapy, obtained thus far in 2 patients with adrenal hyperplasia and hypertension (M.C.S. and J.J.); and c) curves with a marked peak at 600 mμ, which is not reduced by cortisone therapy, obtained in cases of virilizing or hirsutizing adrenocortical tumor.

Effects of cortisone on urinary steroid excretion. In all 16 cases of congenital adrenal hyperplasia treated with cortisone in this clinic there has been a suppression of urinary 17-ketosteroid excretion. In none of the cases of adrenal tumor which have been described was there suppression of urinary 17-ketosteroid excretion by cortisone.

In the 2 patients (M.C.S. and J.J.) with adrenal hyperplasia and hypertension who had slightly increased excretions of DHIA steroids, the administration of cortisone was associated with a fall in excess 17-ketosteroid excretion, the disappearance of DHIA steroids from the urine and a fall in blood pressure to normal values.

The 2 patients with virilizing adrenal tumor (H.B. and M.B.) and the 1 patient with hirsutizing adrenal tumor (E.H.) showed considerably elevated urinary excretions of 17-ketosteroids and DHIA steroids, neither of which was reduced by cortisone therapy (Figs. 3, 5 and 7). When cortisone was given to Patient H. B. the temporary fall in urinary 17-ketosteroids was mostly due to a decrease in the DHIA steroid moiety. Both Patients M.B. and E.H. showed additional increases in their 17-ketosteroid ex-

cretion when cortisone was given (Figs. 3 and 7). In Patient M.B. this increase seemed to consist of non-DHIA 17-ketosteroids, whereas in Patient E.B. the increase was in DHIA steroids. The patient with adrenal tumor and Cushing's syndrome (H.G.) excreted abnormal quantities of 17-ketosteroids but no measurable DHIA steroids (Fig. 9). There was no diminution of urinary 17-ketosteroid output when cortisone was given.

In the 3 cases of adrenal tumor which were explored, all showed postoperatively a fall of DHIA steroid excretion to values too low to determine, and a fall of urinary 17-ketosteroid excretion to normal levels.

Numerous studies have been made in an effort to elucidate the cause of the increased excretion of dehydroisoandrosterone in cases of adrenal tumor (27–33). When dehydroisoandrosterone was administered to adults, the resulting increased 17-ketosteroid excretion was found to be principally composed of androsterone and etiocholanol-3(alpha)-one-17, indicating that some *in vivo* conversion of beta to alpha configuration took place (29–31). *In vitro* studies indicated that liver tissue converted dehydroisoandrosterone to Δ^5-androstene-3(beta),17(alpha)-diol and Δ^5-androstene-3(beta),16(beta),17(alpha)-triol (32). Thus it was suspected by Mason and colleagues that the excessive excretion of dehydroisoandrosterone in cases of virilizing adrenal tumor might be the result of overwhelming the normal mechanisms for converting dehydroisoandrosterone to other steroids. This hypothesis they tested by administering 150 mg. per day of dehydroisoandrosterone acetate to a patient with a virilizing adrenal tumor, both before and after operation (33). To their surprise, there appeared to be a more effective conversion of the administered steroid to other products *preoperatively* than after the tumor was removed.

Cortisone has been shown to activate experimental lymphosarcoma in rats by suppressing immune bodies normally developed against the tumor (34). Whether the increased 17-ketosteroid and DHIA steroid excretion during cortisone therapy in our adrenal tumor cases represented activation of tumor metabolism due to cortisone, or direct conversion of administered cortisone into 17-ketosteroids (35, 36) is a question not answered by the data presented.

Effects of cortisone on excretion of reducing corticoids. All 4 patients with adrenocortical tumor excreted increased amounts of reducing corticoids prior to cortisone therapy. Patient H.B. showed a marked rise of reducing corticoid excretion during the first two days of cortisone therapy. This occurred (Fig. 5) just when urinary DHIA steroid excretion fell. When the DHIA excretion again increased on the fourth day of cortisone the reducing corticoid excretion dropped. Other data indicate that urinary fluorometrically measured estrogens followed a pattern similar to that of the reducing corticoids (37).

These transitional changes may represent a delay in adaptation of hepatic or renal pathways of cortisone metabolism. The temporal lag in adaptation of bacterial cultures to the utilization of a new and unfamiliar substrate is well documented (38).

Effects of ACTH on urinary steroid excretion. The effects of ACTH administration to patients with congenital adrenal hyperplasia have been previously reported (39, 12).

There was no appreciable effect of ACTH on urinary 17-ketosteroids or DHIA steroids in the 2 patients with adrenal tumor (M.B and H.B.; Figs. 3 and 5, Table 1). There was no effect of ACTH on urinary reducing corticoids in M.B., but in Patient H. B. there was a sharp rise. This lack of influence of ACTH on excretion of 17-ketosteroids and DHIA steroids is cases of adrenal tumor is not surprising. Observations by Landau and colleagues suggest that the normal adrenal cortex does respond to ACTH stimulation by secreting increased dehydroisoandrosterone as well as other 17-ketosteroids. These workers showed that the urinary excretion of ketonic Pettenkofer chromogens parallels 17-ketosteroid excretion under ACTH stimulation in the normal adult (40).

Value of these studies in differential diagnosis of adrenal tumors. It is important to differentiate congenital adrenal hyperplasia from adrenocortical tumor with a minimum of delay, since treatment of the former is medical (11–19) and treatment of the latter is surgical. If postnatal virilization has occurred in a child under 10 years of age, tumor should be strongly suspected (41). Females with congenital adrenal hyperplasia usually offer little difficulty in differential diagnosis due to the presence of a urogenital sinus or enlarged clitoris at birth, but in males with congenital adrenal hyperplasia virilization may not be apparent.

From the foregoing data, it would appear that two new procedures are

TABLE 3. COMPARISON OF 17-KETOSTEROID AND DHIA STEROID EXCRETION IN CONGENITAL ADRENAL HYPERPLASIA, VIRILIZING ADRENOCORTICAL TUMOR AND CUSHING'S SYNDROME, SHOWING THE DIFFERENTIAL SUPPRESSIVE EFFECT OF CORTISONE IN THESE THREE GROUPS OF CASES

	Urinary steroid excretion		Suppression of urinary steroids by cortisone	
	17-KS	DHIA	17-KS	DHIA
Congenital adrenal hyperplasia	+++	N or +	Yes	Yes
Virilizing adrenocortical tumor	++++	++++	No	No
Cushing's syndrome	+ or +++	N or ?+	No	—

now available to aid in differentiating between congenital adrenal hyperplasia and adrenocortical tumor: a) suppression of increased 17-ketosteroid excretion by cortisone therapy, and b) colorimetric estimation of increased dehydroisoandrosterone excretion (1, 9, 10, 42–44) and its response to cortisone therapy. Table 3 shows the response of various forms of hyperadrenocorticism to these tests. The finding of an increased excretion of urinary 17-ketosteroids and DHIA steroids, neither of which is decreased by cortisone treatment, is very strong evidence in favor of adrenocortical tumor. Patients with Cushing's syndrome may have increased 17-ketosteroid excretion unresponsive to cortisone therapy and yet excrete no excess DHIA steroids. It must be emphasized that a negative finding should never suffice to exclude an adrenal tumor.

SUMMARY

1. Two cases of virilizing adrenocortical tumor were studied preoperatively with ACTH and cortisone. Measurements of urinary DHIA steroids, 17-ketosteroids and reducing corticoids were made, together with spectral absorption curves of the DHIA reaction. ACTH therapy caused no further increase in the urinary excretion of DHIA steroids and 17-ketosteroids. In one case cortisone therapy was associated with an increase of 17-ketosteroid excretion; in the other case cortisone caused only a transitory decrease in the excretion of the DHIA steroid fraction. In both cases there was a postoperative fall of urinary steroids to normal values and disappearance of the peaks at 600 mμ in the spectral curves.

2. One case of hirsutizing adrenocortical tumor was studied in a similar manner. Cortisone therapy was associated with an additional rise in urinary DHIA steroid excretion. Postoperatively there was a fall of the measured steroids to normal values and disappearance of the peak at 600 mμ in the spectral curve.

3. One case of adrenocortical tumor of the Cushing's syndrome type was studied. Cortisone therapy produced no measurable changes in urinary excretion of 17-ketosteroids or reducing corticoids.

4. Seven patients with adrenocortical virilism and without neoplasms or Cushing's syndrome were studied. All these patients showed a fall of 17-ketosteroid excretion to normal values as a result of treatment with cortisone or compound F. Three of these patients showed spectral curves which were not modified by cortisone therapy. One patient showed no modification of the spectral curve during compound F therapy, and 1 patient showed no such change during ACTH therapy. Two patients who also had hypertension exhibited spectral curves with moderate peaks at 600 mμ. When the latter 2 patients were treated with cortisone both the hypertension and the peaks at 600 mμ were diminished.

5. The value of these studies in the differential diagnosis of adrenal tumors is discussed.

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REFERENCES

1. Allen, W. M.; Hayward, S. J., and Pinto, A.: A color test for dehydroisoandrosterone and closely related steroids, of use in the diagnosis of adrenocortical tumors, *J. Clin. Endocrinol.* **10:** 54, 1950.
2. Callow, R. K.: Isolation of the male hormone present in the urine of a patient with an adrenal tumor, *J. Soc. Chem. Ind.* (London) **55:** 1030, 1936.
3. Talbot, N. B.; Butler, A. M., and MacLachlan, E. A.: Alpha and beta neutral ketosteroids (androgens), *New England J. Med.* **223:** 369, 1940.
4. Crooke, A. C., and Callow, R. K.: The differential diagnosis of forms of basophilism (Cushing's syndrome) particularly by the estimation of urinary androgens, *Quart. J. Med.* n.s. **8:** 233, 1939.
5. Wolfe, J. K.; Fieser, L. F., and Friedgood, H. B.: Nature of the androgens in female adrenal tumor urine, *J. Am. Chem. Soc.* **63:** 582, 1941.
6. Frame, E. G.: A micro method for the separation of 17-ketosteroids into alpha and beta fractions, *Endocrinology* **34:** 175, 1944.
7. Mason, H. L., and Kepler, E. J.: Isolation of steroids from the urine of patients with adrenal cortical tumors and adrenal cortical hyperplasia, *J. Biol. Chem.* **161:** 235, 1945.
8. Dirscherl, W., and Zilliken, F.: Eine Farbreaktion des trans-dehydro Androsterone, *Naturwissenschaften* **31:** 349, 1943.
9. Patterson, J.: Diagnosis of adrenal tumours: a new chemical test, *Lancet* **2:** 580, 1947.
10. Jensen, C. C.: Quantitative determination of dehydroisoandrosterone, *Acta endocrinol.* **4:** 140 and 374, 1950.
11. Wilkins, L.; Lewis, R. A.; Klein, R., and Rosemberg, E.: The suppression of androgen secretion by cortisone in a case of congenital adrenal hyperplasia, *Bull. Johns Hopkins Hosp.* **86:** 249, 1950.
12. Bartter, F. C.; Forbes, A. P., and Leaf, A.: Congenital adrenal hyperplasia associated with the adrenogenital syndrome: an attempt to correct its disordered hormonal pattern, (abstract) *J. Clin. Investigation* **29:** 797, 1950 and **30:** 237, 1951.
13. Wilkins, L.; Lewis, R. A.; Klein, R., and Rosemberg, E.: Die Wirkung von Cortison auf die Ausscheidung der 17-Ketosteroide und anderer Steroide bei Patienten mit kongenitaler Nebennierenhyperplasie, *Helvet. paediat. acta* **5:** 418, 1950.
14. Prader, A.: Pseudohermaphroditismus femininus mit kongenitaler Nebennierenrinden-Insuffizienz, *Helvet. paediat. acta* **5:** 426, 1950.
15. Wilkins, L.; Lewis, R. A.; Klein, R.; Gardner, L. I.; Crigler, J. F., Jr.; Rosem-

BERG, E., and MIGEON, C. J.: Treatment of congenital adrenal hyperplasia with cortisone, *J. Clin. Endocrinol.* **11:** 1, 1951.

16. WILKINS, L.; GARDNER, L. I.; CRIGLER, J. F., JR., and MIGEON, C. J.: Treatment of congenital adrenal hyperplasia with cortisone: comparison of intramuscular and oral dosage, *Tr. A. Am. Physicians* **64:** 160, 1951.
17. WILKINS, L.; GARDNER, L. I.; CRIGLER, J. F., JR.; SILVERMAN, S. H., and MIGEON, C. J.: Further studies on the treatment of congenital adrenal hyperplasia with cortisone. I. Comparison of oral and intramuscular administration of cortisone, with a note on the suppressive action of compounds F and B on the adrenal, *J. Clin. Endocrinol. & Metab.* **12:** 257, 1952.
18. WILKINS, L.; CRIGLER, J. F., JR.; SILVERMAN, S. H.; GARDNER, L. I., and MIGEON, C. J.: *Id.* II. The effects of cortisone on sexual and somatic development, with an hypothesis concerning the mechanism of feminization, *Ibid.*, p. 277.
19. WILKINS, L.; CRIGLER, J. F., JR.; SILVERMAN, S. H.; GARDNER, L. I., and MIGEON C. J.: *Id.* III. The control of hypertension with cortisone, with a discussion of variations in the type of congenital adrenal hyperplasia and report of a case with probable defect of carbohydrate-regulating hormones, *Ibid.*, p. 1015.
20. VENNING, E.: Comments *in* Proceedings of the Second ACTH Conference (Chicago, 1950), edited by John R. Mote. New York, The Blakiston Company, 1951, vol. 2, pp. 91–92.
21. ALLEN, W. M.: A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids, *J. Clin. Endocrinol.* **10:** 71, 1950.
22. CORCORAN, A. C.: Nomenclature in chemical adrenal steroid assays, *J. Clin. Endocrinol.* **11:** 445, 1951.
23. HEARD, R. D. H., and SOBEL, H.: VIII. A colorimetric method for the estimation of reducing steroids, *J. Biol. Chem.* **165:** 687, 1946.
24. CALLOW, N. H., and CALLOW, R. K.: The isolation of androsterone and transdehydroandrosterone from the urine of normal women, *Biochem. J.* **32:** 1759, 1938.
25. BITMAN, J., and COHEN, S. L.: Hydrolysis of urinary conjugated 17-ketosteroids by acetate buffer, *J. Biol. Chem.* **191:** 35, 1951.
26. TALBOT, N. B.: RYAN, J., and WOLFE, J. K.: The determination of sodium dehydroisoandrosterone sulfate in water or urine, *J. Biol. Chem.* **148:** 593, 1943.
27. DORFMAN, R. I., and HAMILTON, J. B.: Concerning the metabolism of testosterone to androsterone, *J. Biol. Chem.* **133:** 753, 1940.
28. FRAME, E. G.; FLEISCHMANN, W., and WILKINS, L.: The influence of a number of androgenic steroids on the urinary excretion of the neutral 17-ketosteroids, *Bull. Johns Hopkins Hosp.* **75:** 95, 1944.
29. MASON, H. L., and KEPLER, E. J.: Isolation of androsterone, etiocholan-3(α)-ol-17-one and Δ^5-androstene-3(β),17(α)-diol from the urine after administration of dehydroisoandrosterone to a man, *J. Biol. Chem.* **160:** 255, 1945.
30. MASON, H. L., and KEPLER, E. J.: Urinary steroids isolated after administration of dehydroisoandrosterone to human subjects, *J. Biol. Chem.* **167:** 73, 1947.
31. MILLER, A. M.; DORFMAN, R. I., and MILLER, M.: Metabolism of the steroid hormones: metabolism of dehydroisoandrosterone, *Endocrinology* **46:** 105, 1950.
32. SCHNEIDER, J. J., and MASON, H. L.: Studies on intermediary steroid metabolism: I. Isolation of Δ^5-androstene-3(β),17(α)-diol and Δ^5-androstene-3(β),16(β),17(α)-triol following the incubation of dehydroisoandrosterone with surviving rabbit liver slices, *J. Biol. Chem.* **172:** 771, 1948.

33. Mason, H. L.; Kepler, E. J., and Schneider, J. J.: Metabolism of dehydroisoandrosterone in a woman before and after removal of an adrenocortical tumor, *J. Biol. Chem.* **179:** 615, 1949.
34. Stoerck, H. C.: Suppression of tumor "immunity" by cortisone and following pyridoxine deprivation. Presented before the American Chemical Society, October 28–29, 1950.
35. Sprague, R. G.; Kendall, E. D.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **8:** 199, 1950.
36. Thorn, G. W.; Forsham, P. H.; Bennett, L. L.; Roche, M.; Reiss, R. S.; Slessor, A.; Flink, E. B., and Somerville, W.: Clinical and metabolic changes in Addison's disease following the administration of compound E acetate, *Tr. A. Am. Physicians* **62:** 233, 1949.
37. Migeon, C. J., and Gardner, L. I.: Urinary estrogens (measured fluorometrically and biologically) in hyperadrenocorticism: influence of cortisone, compound F, compound B and ACTH, *J. Clin. Endocrinol. & Metab.* **12:** (in press) 1952.
38. Monod, J.: The growth of bacterial cultures. *Ann. Rev. Microbiol.* **3:** 371–394, 1949.
39. Lewis, R. A., and Wilkins, L.: The effect of adrenocorticotrophic hormone in congenital adrenal hyperplasia with virilism and in Cushing's syndrome treated with methyl testosterone, *J. Clin. Investigation* **28:** 394, 1949.
40. Landau, R. L.; Knowlton, K.; Lugibihl, K., and Kenyon, A. T.: The effect of adrenal stimulation with adrenocorticotrophic hormone on the excretion of dehydroisoandrosterone in man, *Endocrinology* **48:** 489, 1951.
41. Wilkins, L.: A feminizing adrenal tumor causing gynecomastia in a boy of five years contrasted with a virilizing tumor in a five-year-old girl: classification of seventy cases of adrenal tumor in children, *J. Clin. Endocrinol.* **8:** 111, 1948.
42. Munson, P. L.; Jones, M. E.; McCall, P. J., and Gallagher, T. F.: A colorimetric method for estimation of dehydroisoandrosterone and its application to urinary extracts, *J. Biol. Chem.* **176:** 73, 1948.
43. Hansen, L.: A modified Pettenkofer reaction for the quantitative estimation of dehydroisoandrosterone and its application to analysis of urinary extracts and fractionations, *Endocrinology* **44:** 492, 1949.
44. Gardner, L. I., and Migeon, C. J.: Le diagnostic des tumeurs virilisantes du cortex surrénalien, *Helvet. paediat. acta,* **6:** 465, 1951.

METABOLIC STUDIES IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA. EFFECTS OF CORTISONE THERAPY*

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ADRENAL hyperplasia may be congenital or acquired and may occur in either male or female. In the female it may manifest itself as female pseudohermaphrodism or as virilism. If the onset of hyperplasia occurs in utero between the third and fifth fetal month, as is the usual case, female pseudohermaphrodism results (1). If the onset is at a later gestational period or postnatally, only virilism results, since the differentiation of the urogenital sinus has occurred before this time. In the male if the onset of adrenal hyperplasia occurs either in utero or before the age of puberty, macrogenitosomia praecox results. All recorded cases of the adrenogenital syndrome with onset after birth but before the age of 10 years have been due to tumor rather than to adrenal hyperplasia (2).

In common with other poorly understood medical conditions, congenital adrenal hyperplasia has been approached therapeutically by a number of widely divergent regimens. The results of surgical extirpation of adrenal cortical tissue in general have been disappointing (1). Similarly, therapy with various hormonal preparations, until recently, has met with little success (1, 3, 4). However, during the past two years, since the introduction of cortisone therapy by Wilkins and coworkers (3, 5, 6), the approach to treatment of patients with this condition has been revolutionized.

The purpose of this paper is to report a variety of metabolic studies which have been made in 4 patients with congenital adrenal hyperplasia. These studies were performed before and during periods of cortisone therapy and after temporary interruption of therapy, in an attempt to improve the understanding of the basic physiologic mechanisms involved, as well as to evaluate the influence of cortisone therapy on these patients.

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MATERIALS AND METHODS

The patients used in this study were 4 children with congenital adrenal hyperplasia; of these, 3 had female pseudohermaphrodism and 1, macrogenitosomia praecox. These children ranged in age from $2\frac{1}{2}$ to $5\frac{3}{4}$ years when observations were begun on them at this hospital. All had been observed or treated elsewhere previously.

The 24-hour urinary 17-ketosteroid excretions were measured by the method of Callow, Callow and Emmens (7 a) and by a modification of the method of Cahen and Salter (7 b). Electrolyte determinations included: chlorides by the method of Volhard (8); sodium and potassium, by flame photometry[1] and true plasma bicarbonate by the manometric method of Van Slyke (9). The plasma pH was measured by the electrometric method.

In the eosinophil response studies the counts of absolute numbers of eosinophils (by the method of Randolph (10)) were made prior to, and one, two and four hours after the administration of epinephrine or ACTH. Epinephrine hydrochloride was injected intramuscularly, 0.01 mg./Kg. of body weight. ACTH (ACTHAR®) was injected intramuscularly in 25-I.U. or 100-I.U. amounts. Glucose determinations were carried out on the same blood samples and, in addition in some instances, at forty minutes after the administration of epinephrine or ACTH, by the micromethod of Nelson (11). However, only the control and one-hour values are discussed in this presentation.

The levels of 17-hydroxycorticosteroids in whole blood were determined by the method of Nelson and Samuels and these levels in plasma were determined by a modification of this method (12).

The general plan of study and evaluation of therapy in these patients has been divided into four phases: 1) the pre-therapy period; 2) the period of initial (maximal) cortisone therapy; 3) the period of maintenance cortisone therapy; and 4) the period following temporary discontinuation of cortisone therapy. The initial cortisone therapy was 50 mg. of cortisone acetate intramuscularly per day. Maintenance doses were individualized and dependent upon the response of each patient to therapy.

OBSERVATIONS

All of the children with congenital adrenal hyperplasia exhibited advanced somatic growth and excessive masculinization. Table I shows the chronologic age and in comparison with this, in each patient, the advanced bone age, height age, and weight age. This physical precocity is particularly striking in Patients 3 and 4. The mental age is appropriate to the

[1] Performed through the courtesy of Dr. Virginia Davenport, Department of Physiology, University of Utah College of Medicine.

TABLE 1. DEVELOPMENTAL STATUS OF PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

Patient	Diagnosis	Age	Bone age	Height age	Weight age	Mental age
1	F. P.* explored	2 yrs., 6 mos.	5– 7 yrs.	3¼ yrs.	3½ yrs.	2 yrs., 3 mos.
2	M. G.†	4 yrs., 1 mo.	8– 9 yrs.	5¼ yrs.	5¼ yrs.	4 yrs., 1 mo.
3	F. P.* explored	4 yrs., 5 mos.	11–12 yrs.	8½ yrs.	11¼ yrs.	4 yrs., 8 mos.
4	F. P.* explored	5 yrs., 9 mos.	15–17 yrs.	9½ yrs.	10½ yrs.	7 yrs., 5 mos.

* Female pseudohermaphrodite.

† Macrogenitosomia praecox.

Note: Patient 4 had a panhysterectomy, ovariectomy and unilateral subtotal adrenalectomy at the age of 2 years.

chronologic age in Patients 1 and 2, and advanced in Patients 3 and 4. In general, mental age is not particularly affected by this condition.

Figure 1 illustrates the growth curves for these children. As seen in this figure, their respective heights and weights are advanced to various degrees in comparison with the normal population of children of corresponding ages. The rapid increase and decrease in the weights of Patients 1 and 2, indicated by the arrows, reflects side effects of initial cortisone therapy, as will be discussed later.

Case histories

Patient 1, E.W., is shown in Figures 2 a and b. Age, 2 years and 5 months; height, 31 inches; weight, 35 pounds. At birth, the parents were told that the patient was a boy. Exploratory laparotomy, performed at the age of 2 weeks, revealed female internal genitalia; the decision was then made to raise her as a girl. At 4 weeks she was hospitalized because of "colic," weight loss and a renal infection, requiring extensive parenteral fluid therapy. Bone age at 11 months of age was normal. "Tomboyish" behavior was noted shortly after 18 months of age, consisting of boisterousness and aggressiveness. *Family history:* She had 1 sibling (Patient 2, with macrogenitosomia praecox). Family history was otherwise noncontributory. *Physical examination:* Pertinent findings were limited to the laparotomy scar, an enlarged clitoris 2 cm. in length with a hypospadic urogenital sinus opening at its base, and labia majora which had the appearance of a bifid scrotum, Psychologic examination[2] showed an I.Q. of 93 with no evidence of deviant personality development. *Course:* Following base-line metabolic studies, the administration of cor-

[2] Psychologic evaluations of all these patients were performed by Dr. William H. Brown, Department of Psychiatry, University of Utah College of Medicine.

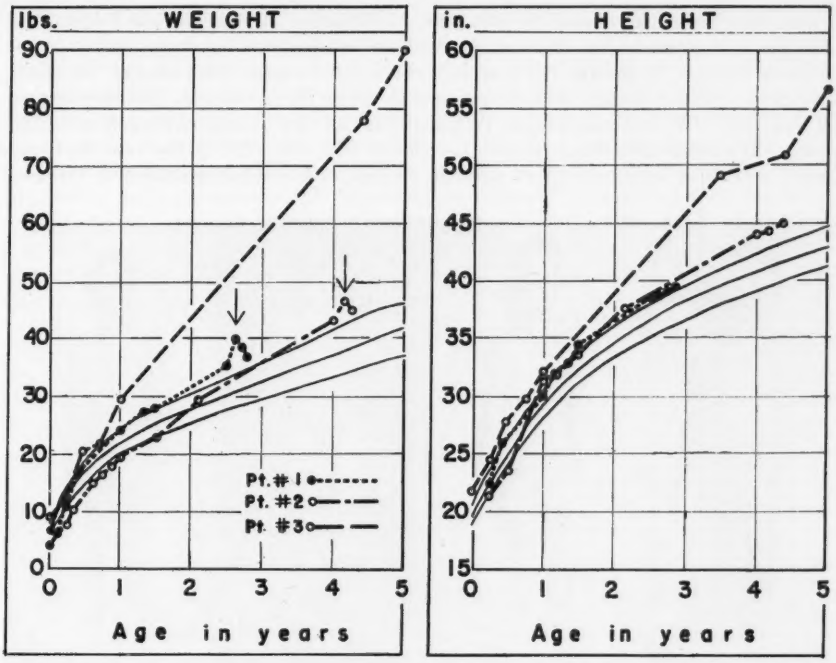

FIG. 1. Growth curves of patients with congenital adrenal hyperplasia. The solid lines represent the mean and mean ± one standard deviation for the normal population. These norms are adapted from Jackson and Kelly (42).

tisone was begun, in a dose of 50 mg. per day. The 17-ketosteroid excretion levels fell precipitously and remained low on this dosage. After eighteen days the dosage was reduced to 25 mg. per day, which was continued for thirty-two days, during which time the 17-ketosteroid excretion levels remained low. However, evidences of undesirable side effects of the therapy developed; these disappeared when the dosage was decreased to 25 mg. every other day, although there was no significant elevation of the 17-ketosteroid levels. After the patient had received this dose for thirty-seven days the cortisone was discontinued temporarily. The aggressive, boisterous behavior, which was present prior to therapy and which had disappeared during therapy, again became noticeable; also, the 17-ketosteroid values began to show elevation toward pre-therapy levels. After a fifty-day period of observation without therapy, cortisone was restarted in a dosage of 25 mg. every other day.

Patient 2, B. W., the sibling of Patient 1, is shown in Figures 2 c and d. Age, 4 years and 1 month; height, 46½ inches; weight, 43½ pounds. This child, born prematurely with a birth weight of 4 pounds, 8 ounces, remained in an incubator with oxygen for six weeks and was discharged at 8 weeks of age. During the first three or four months of life, several transitory, ill-defined cyanotic episodes occurred. The mother observed during the first weeks of life that the child had an exceptionally large penis but was told by the family physician that this was a physiologic variant. "Projectile vomiting" and feeding diffi-

culties were prominent during the first year. At the age of 2½ years, he was hospitalized because of a severe febrile illness which remained unidentified but responded to streptomycin therapy. At the age of 1 year, he was noted to be larger than normal. The penis had grown proportionately, remaining unusually large. *Family history:* (*See* case history of Patient 1.) *Physical examination:* Pertinent findings were limited to the genitalia. The penis was exceptionally large, measuring 6.5 cm. in length. The testes were relatively small, and there were no evident pubertal changes. Psychologic examination revealed

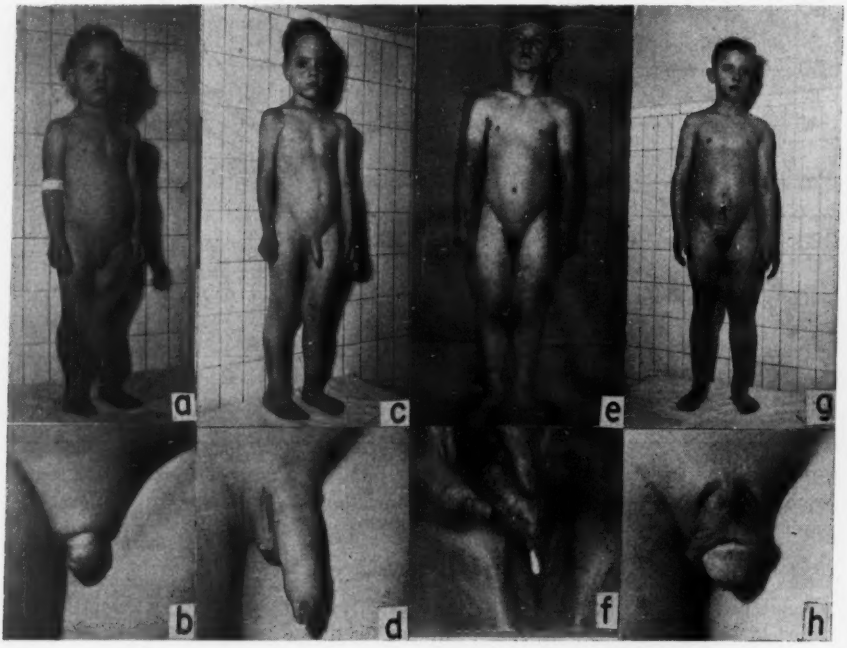

FIG. 2. *a and b:* Patient 1, E. W., female pseudohermaphrodite, age 2½ years. Photo *b* shows the appearance of the external genitalia. Note the enlarged clitoris. *c and d:* Patient 2, B. W., macrogenitosomia praecox, age 4 years and 1 month. Photo *d* shows the enlarged penis. *e and f:* Patient 3, R. W., female pseudohermaphrodite, age 4 years and 5 months. Note evidences of masculinization. Photo *f* shows close-up of genitalia with enlarged clitoris and cotton pledget in the opening of the urogenital sinus. *g and h:* Patient 4, W. C., female pseudohermaphrodite (panhysterectomized, ovariectomized, and unilaterally adrenalectomized), age 5 years and 9 months. Photo *h* showed the external genitalia with the enlarged clitoris. Pubic hair has been shaved.

an I.Q. of 100 and no obvious personality aberration. *Course:* Therapy and study in this patient were carried out simultaneously and identically with those of Patient 1. Similar side effects of cortisone therapy appeared at a corresponding time and disappeared following reduction of the dose, as in Patient 1, and the response of 17-ketosteroid excretion was nearly identical. The most recent physical examination, five months after the initial one, revealed no measurable change in the size of the penis.

Patient 3, R. W., is shown in Figures 2 e and f. Age, 4 years and 5 months; height, 51 inches; weight, 78 pounds. At birth, abnormal genitalia were noted, and although the doctor felt the child was a girl, the parents insisted upon giving it a boy's name. There was some unusual vomiting at 6 weeks of age, but this improved after a short period of time. The patient began to show accelerated growth in infancy, and after this time was always larger than children her own age. At 14 months of age she was treated with pituitary gonadotropin for four months, during which time the "chordee penis" enlarged noticeably. At 3½ years of age, the "chordee" was repaired by plastic surgery. Increased strength became apparent at about this time. Six months later pubic hair was first noted and further enlargement of the "penis" with onset of erections occurred. Facial acne and deepening of the voice appeared shortly thereafter. *Family history:* Noncontributory. The only sibling, a female, was apparently normal. *Physical examination:* She appeared as a tall, muscular, pubertal male with unusual strength. Blood pressure was 110/80 mm. of Hg. Features noted were: mild facial acne, deep voice, downy hair on the cheeks, considerable pubic hair with male distribution, a large phallus having a sizable opening at its base, and hypertrophic labia majora which resembled a bifid scrotum. Psychologic examination showed an I.Q. of 119, with no particular male or female pattern of response. *Course:* X-ray examination with Lipiodal instillation into the urogenital sinus outlined a vagina, uterus, and fallopian tubes. Exploratory laparotomy was performed at the parents' insistence and revealed normal female internal genitalia with bilateral adrenal hyperplasia. A biopsy specimen of the ovary was obtained. Following base-line determinations of urinary 17-ketosteroids, cortisone was started in a dosage of 50 mg. per day, after which the 17-ketosteroid excretion decreased precipitously. When the dose was decreased to 25 mg. per day there was a definite increase in 17-ketosteroid excretion, but it remained significantly below pre-therapy levels. Following a further decrease in the dose to 12.5 mg. per day, another moderate elevation in 17-ketosteroid excretion occurred. At this time daily administration of 25 mg. of cortisone orally was begun; this proved inadequate, so the method of administration was changed back to the intramuscular route. Since that time the patient has received 25 mg. of cortisone intramuscularly per day, with the exception of a few brief periods of unprescribed omission. On this regimen the urinary excretion of 17-ketosteroids has decreased until at the present time it is approximately 6 mg. per day. In spite of the fact that the 17-ketosteroid excretion has not been consistently maintained within normal limits, the child has grown six inches in height without evidence of concomitant advance in bone age during the eighteen months of cortisone therapy.

Patient 4, W. C., is shown in Figures 2 g and h. Age, 5 years and 9 months; height, 53¼ inches; weight 72 pounds. At birth "hypospadias" was noted. The sex remained undecided at first, but after the phallus had enlarged markedly the decision was made at 9 months of age to regard the patient as a male. She was noted to be large, strong and masculine at 1 year of age. At 15 months the appearance of pubic hair and facial acne was noted. At 19 months she was hospitalized elsewhere for study. X-ray examination revealed a bone age of 8 years and a 24-hour 17-ketosteroid excretion was 17.5 mg. Laparotomy performed at that time revealed complete internal female genitalia and bilaterally enlarged adrenals. The left adrenal gland was completely removed; pathologic examination showed hyperplasia. At 4 years of age, she underwent panhysterectomy and bilateral ovariectomy. The following year plastic correction of the hypospadias was attempted. *Family history:* Noncontributory. No siblings. *Physical examination:* B.P., 105/70. The child was large, muscular and of masculine configuration with pubertal changes in the voice. Pertinent findings were limited to the laparotomy scars and the

genitalia. The phallus measured 4 cm. in length; evidence of previous surgery was present, in that the urogenital sinus opened 1 cm. above the base of the phallus. The labia majora had a scrotal appearance. Pubic hair with a masculine distribution was present, but closely shaved. Psychologic examination showed an I.Q. of 130 with no abnormal personality development. *Course:* Following base-line metabolic studies, a dose of 50 mg. of cortisone daily was administered. Urinary 17-ketosteroid excretion levels fell rapidly to normal. After ten days the cortisone dosage was decreased to 25 mg. per day; the 17-ketosteroid excretion levels remained depressed. No evidence of side effects from cortisone therapy was noted.

17-Ketosteroid excretions

The 24-hour urinary excretions of 17-ketosteroids are illustrated in Table 2 and Figure 3. These values are listed in Table 2 for the pre-therapy period, the period during initial cortisone therapy, and the period after one month of maintenance cortisone therapy. The pre-therapy value presented for each of the children is an average of several determinations.

TABLE 2. EFFECTS OF CORTISONE THERAPY ON URINARY 17-KETOSTEROID EXCRETION

Patient	17-Ketosteroids (mg./24 hrs.)		
	Pre-therapy	After initial therapy	After 1 mo. of maintenance therapy
1	2.7	0	0.2
2	3.3	0	0.2
3	51.0	4.0	15.2
4	20.5	1.6	0.36

Although these pre-therapy values range from 2.7 to 51.0 mg. per day, they are all in excess of the normal range for the respective ages. The average excretion of these steroids for the first ten days after initiation of cortisone therapy in a dosage of 50 mg. per day was in each case strikingly less than the pre-therapy excretion. For the first twenty-four hours of this therapy these values were: for Patient 1, 0.82 mg.; for Patient 2, 0.54 mg; for Patient 3, 1.2 mg.; and for Patient 4, 4.53 mg. This illustrates the rapid depression of 17-ketosteroid excretion. After one month of maintenance cortisone therapy of 25 mg. per day the values remained well below the pre-therapy levels.

Figure 3A shows the 17-ketosteroid excretions of Patients 1 and 2, illustrating the continued effect of the decreasing cortisone doses and the increase in these excretion values when cortisone was discontinued temporarily. Patient 4, whose values are shown in Figure 3B, responded to cortisone in much the same manner as did Patients 1 and 2; at the present

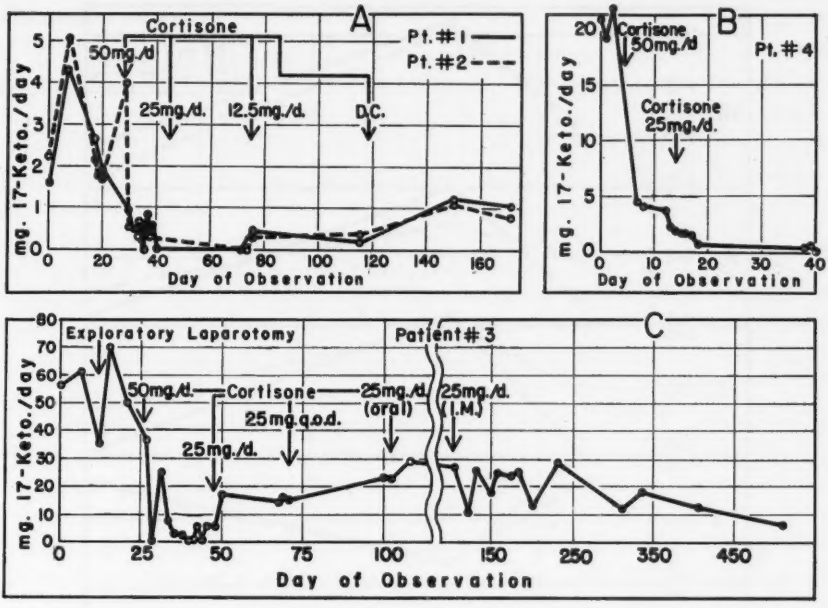

FIG. 3. Urinary 17-ketosteroid excretion in patients with congenital adrenal hyperplasia. Response to cortisone therapy. All cortisone doses were administered intramuscularly unless otherwise indicated.

time this patient has been on maintenance therapy for only one month. Figure 3C similarly illustrates the effects of prolonged cortisone maintenance therapy in depressing the 17-ketosteroid excretion levels of Patient 3; although the daily excretion of these steroids varied considerably during the period of maintenance cortisone therapy, at no time did it approach the pre-therapy value. After eighteen months of nearly continuous cortisone therapy the daily urinary excretion of 17-ketosteroids was 6 mg. per day, as compared to the pre-therapy value of 51 mg. per day.

Electrolyte levels

The responses of the plasma pH and certain electrolytes to initation and discontinuation of cortisone therapy are shown in Figure 4, for Patients 1 and 2. The uniformity of the patterns exhibited by these 2 patients is of interest, although it must be emphasized that because of the small numbers of determinations no generalization should be made. In the pre-therapy period the sodium and potassium values were normal, the chloride value was high, the bicarbonate tended to be low and the pH was not elevated. After initial cortisone therapy the sodium, potassium, bicar-

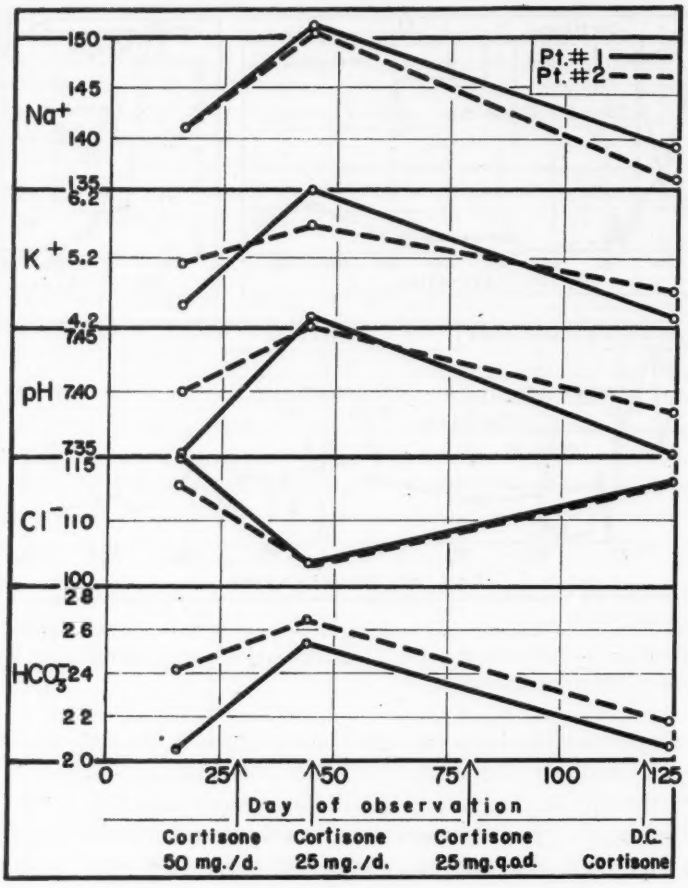

FIG. 4. Effects of cortisone therapy on electrolytes.

bonate and pH values all rose, whereas the chloride returned to normal. During the period when cortisone was discontinued, after about two and a half months of maintenance cortisone therapy, these values all reverted to the pre-therapy ranges. Patient 4 also had pre-therapy values similar to those shown for Patients 1 and 2, but no further values have been obtained as yet.

It is of interest to note that these 3 children all had high initial chloride levels, which returned to normal during cortisone therapy in Patients 1 and 2 and then rose after cortisone was discontinued. Also of interest is the sodium-chloride dissociation and the parallelism of sodium and potassium values.

Eosinophil responses

In Figure 5 are illustrated the eosinophil responses in Patients 1 and 2 to the injection of ACTH in doses of 25 and 100 I.U. and of epinephrine, before and during the administration of cortisone. The cortisone dose schedule in these 2 patients was as follows: 50 mg. per day for fifteen days followed by 25 mg. per day for one month and then by 25 mg. every other day for forty days. The response to epinephrine injection, shown by the solid lines, was within normal limits for all periods in Patient 1. However, in Patient 2 there was a progressive delay in the onset of the eosinophil response, and the eventual result was a negligible response after cortisone had been administered in a dose of 25 mg. every other day for forty days. Eosinophil responses to epinephrine in Patient 4 were within normal limits both before and during cortisone therapy.

Eosinophil responses to injections of 25 I.U. of ACTH were grossly abnormal in the pre-therapy period for both Patients 1 and 2, consisting of a marked increase rather than the standard decrease in the eosinophil count. Failure to observe eosinopenia following ACTH injection has been reported previously (13). During the period of maximum therapy in each child these response curves approached normal; however, as the dose of

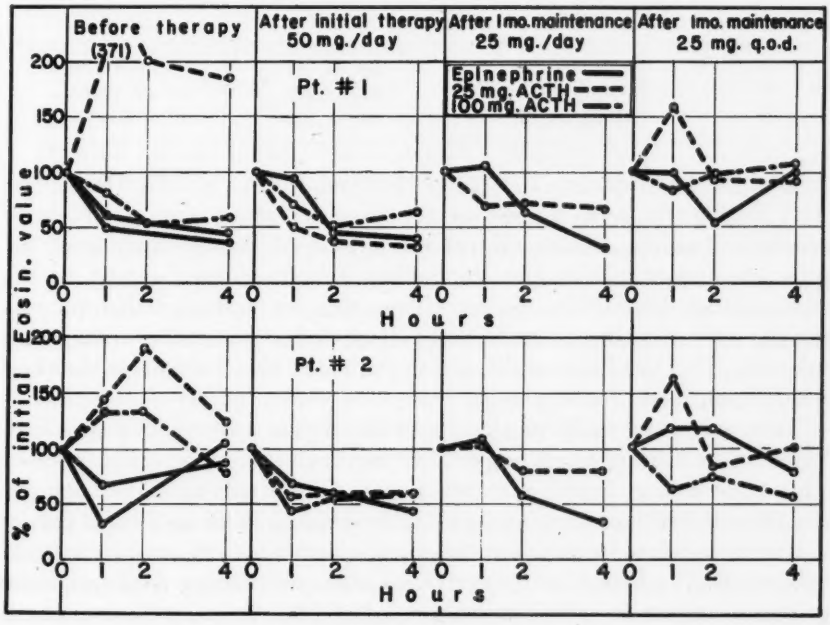

FIG. 5. Influence of cortisone therapy on eosinophil response.

cortisone was decreased progressively the response curves returned toward their initial form. The general pattern of eosinophil responses to 100 I.U. of ACTH was similar to, but not as aberrant as, the pattern of responses to 25 I.U. There was no adequate eosinophil response in Patient 4 to injection of either 25 I.U. or 100 I.U. of ACTH in the pre-therapy or in the cortisone maintenance period.

Glucose responses

The fasting blood sugar levels are shown in Table 3 for Patients 1, 2 and 4. These levels were found to be uniformly in the low-normal or hypoglycemic ranges. During the period of maintenance cortisone therapy the blood sugar levels were lower than the levels in the pre-therapy period for all 3 children and lower than the levels in the post-therapy period for Patients 1 and 2. In spite of these low blood sugar levels none of these children had at any time clinical symptomatology attributable to hypoglycemia.

TABLE 3. EFFECTS OF CORTISONE THERAPY ON FASTING BLOOD SUGAR LEVELS

Patient	Fasting blood glucose (mg./100 cc.)		
	Pre-therapy period	Maintenance therapy period	Post-therapy period
1	60	38, 35, 30	49, 47, 50
2	51, 55	36, 34, 35	42, 37, 65
3	61, 42, 48, 50	41, 46, 40	— — —

The glucose responses to epinephrine and to 25 I.U. and 100 I.U. of ACTH (Fig. 6) were studied in the periods before, during and after cortisone therapy. During the pre-therapy period, by one hour after the injection of epinephrine the glucose levels had returned almost to the fasting level. During the period of maintenance cortisone therapy, the glucose levels remained considerably elevated one hour after epinephrine injection. This was likewise the case in Patients 1 and 2 after cortisone had been discontinued for fifty days.

There were essentially no changes of blood glucose levels in response to 25 I.U. of ACTH before, during or after cortisone therapy in these children. This same lack of response to 100 I.U. of ACTH was noted in both the pre-therapy and post-therapy periods. However, in Patients 1 and 2 particularly, in response to this dose the glucose level was considerably elevated by the end of an hour in the period of maintenance therapy. Although these responses appear to exhibit a definite pattern, the data are inadequate to permit definitive interpretation.

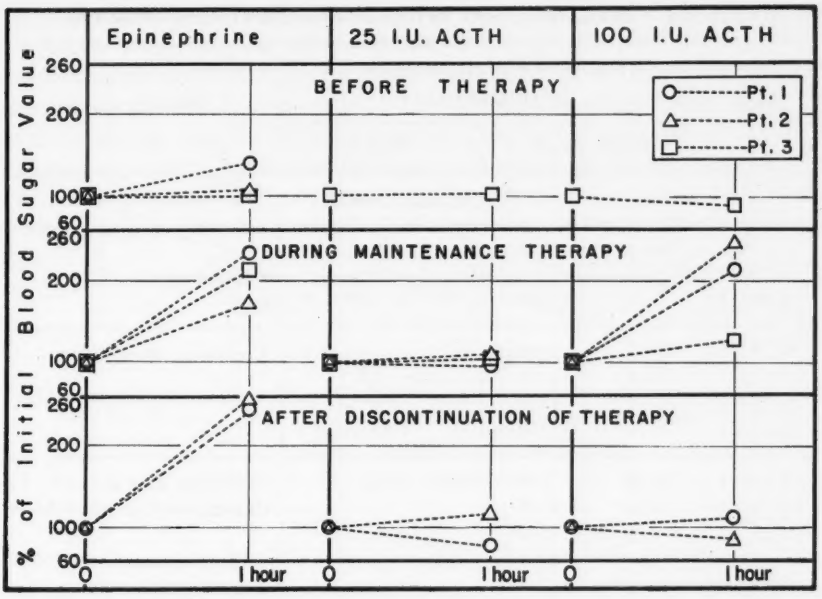

FIG. 6. Effects of cortisone therapy on one-hour glucose responses to epinephrine and ACTH. The lines connecting the points representing the fasting and the one-hour post-injection glucose values are not intended to depict the actual pattern of these responses.

17-Hydroxycorticosteroids

Data on the circulating blood levels of 17-hydroxycorticosteroids are presented in Table 4 for Patients 1, 2 and 4. Values are quoted for both whole blood and plasma, because earlier levels in these children were obtained by the whole blood method, which was later modified to a plasma determination. At the present stage of development of these methods it would seem unjustified to attempt by mathematical manipulation to express the whole blood values in these children in terms of their plasma equivalents.

During the period before cortisone therapy, the 17-hydroxycorticosteroid levels observed in these children appeared to be low or low-normal, and definitely were not elevated. During the entire period of cortisone administration, the levels were elevated compared to the pre-cortisone and post-cortisone periods, probably reflecting measurement of the exogenous cortisone given. (Values were not obtained on Patient 4 during cortisone therapy.) It is evident from the table that for several days after discontinuation of cortisone administration these values remained elevated (except for Patient 4, whose two values were low), but after approximately

TABLE 4. CIRCULATING BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS

Date	17-Hydroxycorticosteroids (micrograms %)				Therapy status
	Whole blood		Plasma		
	Patients 1 and 2				
	Pt. 1	*Pt. 2*			
8/20/51	1.2	2.3			Pre-therapy
8/22/51	3.7	3.0			
9/14/51	10.3	11.4			Cortisone, 50 mg./day
9/19/51	16.7	16.6			
9/24/51	13.5	14.9			
9/25/51	11.0	13.9			
12/11/51	11.6	7.9			Cortisone, 25 mg. q.o.d., was
12/13/51	7.9	11.9			discontinued on 12/9/51
12/15/51	6.4	7.0			
1/14/52	3.2	3.4			1 month off cortisone
			Pt. 1	*Pt. 2*	
2/ 6/52			4.1	2.5	50 days off cortisone
2/ 9/52			1.6	4.7	
	Patient 4				
1/11/52	1.8				Pre-therapy
1/12/52	2.0				
2/19/51			2.0		Cortisone discontinued for 2
2/21/52			0		days

Values for 17-hydroxycorticosteroid blood levels range as follows for normal individuals:

For whole blood, on 10 adults,* 4–10 μg. %
For plasma, on about 50 adults,* 5–20 μg. %
For plasma, on 9 children 4–18 μg. %

* Performed by Dr. Don H. Nelson, Department of Biochemistry, University of Utah College of Medicine.

one month without cortisone therapy they returned to pre-therapy levels.

The response of the 17-hydroxycorticosteroid concentration to a single injection of either cortisone, epinephrine, 25 I.U. of ACTH, or 100 I.U. of ACTH is presented in Table 5. Following an injection of 50 mg. of cortisone intramuscularly, an elevation in the measured 17-hydroxycortico-

steroids appeared in one or two hours in all the children; then the concentration of these steroids slowly returned toward the pre-injection level. There was no significant alteration in concentration of 17-hydroxycorticosteroids in the blood in response to the injection of epinephrine or ACTH in either dosage used in Patients 1 and 2. This lack of response was evident before and during cortisone therapy, and after its discontinuation. In Patient 4 there was also no response in the pre-therapy period. In this patient, after the period of maintenance cortisone therapy, the lack of re-

TABLE 5. RESPONSE OF 17-HYDROXYCORTICOSTEROID BLOOD LEVELS TO EPINEPHRINE, ACTH, AND CORTISONE

Date	17-Hydroxycorticosteroids (micrograms %)				Therapy status
	Whole blood		Plasma		
Patients 1 and 2					
	Pt. 1	*Pt. 2*			
9/24/51 Epinephrine					
0 hour	13.5	14.9			On 50 mg. of cortisone/day.
1 hour	10.5	15.5			
9/25/51 25 mg. ACTH					
0 hour	11.0	13.9			
1 hour	5.4	11.1			
9/26/51 100 mg. ACTH					
0 hour	—	7.7			
1 hour	4.9	6.5			
12/11/51 Epinephrine					
0 hour	11.6	7.9			Cortisone, 25 mg. q.o.d., was discontinued on 12/9/51.
1 hour	11.0	9.3			
12/13/51 25 mg. ACTH					
0 hour	7.9	11.9			
1 hour	6.4	7.8			
12/15/51 100 mg. ACTH					
0 hour	6.2	7.0			
1 hour	7.1	7.3			
			Pt. 1	*Pt. 2*	
2/ 6/52 100 mg. ACTH					
0 hour			4.1	2.5	Cortisone, 50 mg./day, started on 2/9/52.
2 hour			5.1	3.1	
2/ 9/52 Cortisone 50 mg.					
0 hour			1.6	4.7	
2 hours			21.0	24.1	
24 hours			15.7	21.1	

TABLE 5—(*continued*)

Date	17-Hydroxycorticosteroids (micrograms %)		Therapy status
	Whole blood	Plasma	
Patient 4			
1/11/52 25 mg. ACTH			
0 hour	1.8		Therapy
1 hour	2.0		
1/12/52 100 mg. ACTH			
0 hour	2.0		
1 hour	2.5		
1/13/52 Cortisone 50 mg.			
1 hour	7.5		Cortisone, 50 mg./day, started with this injection.
8 hours	3.9		
24 hour	4.3		
2/19/52 Epinephrine			
0 hour		2.0	Cortisone, 25 mg./day, discontinued on 2/18/52.
2 hours		0	
2/21/52 25 mg. ACTH			
0 hour		0	
2 hours		11.3	

sponse to epinephrine was similar to that observed in Patients 1 and 2, but there apparently was an elevation following 25 I.U. of ACTH. This latter response study has not been repeated as yet.

Clinical response to cortisone therapy

Both objective and subjective evaluations of clinical response of these patients to cortisone therapy have yielded suggestive evidence indicating that the therapy is producing its anticipated effects. The only patient who has been maintained on therapy for a period of time long enough to permit meaningful observations of growth (Patient 3) has increased in height from 51 to 57 inches during an eighteen-month period, with no concomitant demonstrable increase in bone age. This same patient has had during this period a striking relief from priapism. Persistent erections, which prior to therapy occurred several times each day, have occurred only rarely since the initiation of treatment. Subjective changes noted in all of these children have consisted of a decrease in strength, in aggressiveness and in irritability, during cortisone therapy.

No decrease in hirsutism or in the size of the phallus has been noted, nor

has there been any evidence of breast development since the initiation of cortisone therapy. There has likewise been no change observed in the deep voice of Patient 3 during the eighteen months of therapy. Psychologic evaluation has revealed no significant change. Undesirable side effects of cortisone which have been observed in these patients include a moderate increase in skin pigmentation in Patient 3, especially in scars, and the development of moon-shaped facies, abdominal distention and obesity in Patients 1 and 2. In these latter 2 patients the side effects mentioned developed while they were receiving 25 mg. of cortisone per day and disappeared after the dose was reduced to 25 mg. every other day.

DISCUSSION

The concept of pathogenesis of this syndrome upon which cortisone therapy is based is essentially as follows: the primary pathology is considered to exist in the adrenal glands, which secrete predominantly a type of hormone incapable of inhibiting pituitary ACTH release. In addition, hormones of the 17-hydroxycorticosteroid type, which are capable of inhibiting the secretion of pituitary adrenocorticotropin, are not present in the blood of such patients in concentrations adequate to exert effective inhibition. ACTH secretion therefore increases, resulting in overstimulation of the adrenal cortex and further overproduction of androgenic hormones. Administration of exogenous cortisone supplements the blood levels of endogenous 17-hydroxycorticosteroids so that levels are obtained sufficient to exert an adequate inhibitory effect on the release of pituitary ACTH.

Cortisone was first employed therapeutically in patients with congenital adrenal hyperplasia by Wilkins and coworkers (3, 5, 6). The rationale advanced by them for the use of cortisone in this syndrome was based on the postulation that administration of this hormone would decrease the secretion of adrenal androgens by suppressing the release of pituitary adrenocorticotropin. The depression of urinary 17-ketosteroid excretion in patients so treated appears to substantiate this hypothesis, especially in view of the finding that the administration of cortisone causes a reduction in the adrenal gland weight of normal rats but not of hypophysectomized rats (14). Conversely, it has been shown that administration of ACTH in this syndrome (13, 15, 16) and of ACTH (17) or epinephrine (18) in normal subjects, results in an elevation of urinary 17-ketosteroids.

Certain data obtained in the present study lend further support to the foregoing hypothesis. The consistent observation of relatively low blood levels of 17-hydroxycorticosteroids in these children prior to therapy is a significant finding in this regard, as is the finding of elevated levels during the periods of cortisone therapy. In addition, preliminary data (19) not presented in this paper suggest that the circulating blood levels of ACTH

in these children prior to therapy are elevated, as compared to those of normal individuals. During the periods of cortisone therapy these levels do not appear to be elevated. It must be emphasized that these are preliminary data and interpretations at this time are only tentative. In adrenalectomized animals the ACTH blood levels are considerably elevated in comparison to those of intact animals (20). This lends support to the theory suggested by Sayers (21, 22) that the anterior pituitary will continue to release ACTH until blood levels of adrenal cortical hormones become sufficient to suppress this release. The existence of relatively low blood levels of 17-hydroxycorticosteroids and relatively high blood levels of ACTH in the period prior to cortisone therapy, together with the reverse situation during the period of cortisone therapy, is in agreement with this theory.

In view of these findings a tentative explanation of the unusual eosinophil response pattern observed following the injection of 25 I.U. of ACTH may be offered. The rise in the eosinophil count observed in response to this dose of ACTH during the pre-therapy period may be considered similar to the epinephrine-eosinophil response in adrenalectomized rats (23). A similar observation has been reported in a child with diabetes insipidus (24). These observations would indicate that the adrenal cortex need not be involved in the eosinophil count elevation in response to stimulation, and suggest that the usual eosinophil response may be a composite result of two forces: one, which is usually dominant, the adrenal cortex, acting to depress the eosinophil count and the other, unknown, acting to elevate it. In the absence of an adequate adrenal response to stimulation this latter force might predominate and the result be a rise in the eosinophil count. If high circulating blood levels of ACTH are present, as seems to be the case in these children before therapy, the injection of 25 I.U. of ACTH may cause a relatively insignificant increment in the circulating ACTH level and, therefore, in the resultant adrenal cortical stimulation. On the other hand, the injection of 100 I.U. of ACTH may represent a significant increment and thus result in a less abnormal eosinophil response pattern. The influence of cortisone therapy upon the eosinophil responses to 25 I.U. of ACTH in these patients lends credence to this explanation. The reduction of ACTH blood levels as a result of pituitary inhibition by cortisone therapy now may make 25 I.U. of ACTH a sufficient increment to effect a normal eosinophil response. As depicted in Figure 5 the eosinophil response to ACTH during the period of maximal cortisone therapy and, therefore, presumably of minimal ACTH blood levels, approaches normal. With progressively decreasing maintenance doses of cortisone the responses tend to revert to their pre-therapy patterns. The general failure of 17-hydroxycorticosteroid blood

levels to become elevated in response to ACTH injection during any period of observation (Table 5) suggests an impaired adrenocortical function of these children. This observation tends to confirm the tentative explanation given for the eosinophil response patterns.

The low-normal fasting blood glucose levels noted in these children (Table 3) may be related to the low 17-hydroxycorticosteroid blood levels. Of interest in this regard is the recent report of symptomatic hypoglycemia in a patient with congenital adrenal hyperplasia (25). It is surprising that during cortisone therapy when the 17-hydroxycorticosteroid blood levels are elevated—a finding which is felt to reflect measurement of the administered cortisone—the fasting blood glucose levels become abnormally low. There seems to be no ready explanation for this.

Although the data presented here are in agreement with the hypothesis proposed (3), certain other possible mechanisms must be considered. Any hypothesis attempting to explain the pathogenesis of congenital adrenal hyperplasia must take into account the following observations which have been reported in this syndrome:

1. The urinary 17-ketosteroid excretion is uniformly elevated. This is a finding commonly interpreted as reflecting excessive production of androgenic hormones by the adrenal cortex (2, 3, 26, 27).
2. This excretion is diminished under the influence of cortisone therapy (3, 5, 6, 15, 28).
3. Frequently such children present clinical evidence of deficiency of electrolyte-regulating hormones (1, 2, 4, 15, 29–33).
4. Deficiency of carbohydrate-regulating hormones may co-exist with overproduction of androgenic hormones in patients with this syndrome (25).
5. On the basis of evidence presented in this report, circulating blood levels of 17-hydroxycorticosteroids appear to be low in patients with this syndrome.
6. From the preliminary work mentioned here, there is a suggestion that these patients have high circulating blood levels of ACTH.

On the basis of these observations it appears unlikely that the primary abnormality is an excessive production of ACTH, since this would not explain the clinical and investigative evidence of simultaneous differential adrenal cortical hormone production. It has been suggested that more than one form of ACTH may be produced by the pituitary (34, 35). The possibility that patients with this sydrome produce a predominance of one form of ACTH resulting in differential adrenal cortical stimulation cannot be ruled out on the basis of the evidence at hand.

The production of a single "mother hormone" has been postulated (36–40). If this were the case the basic abnormality in patients with this syndrome might be a preferential conversion of this "mother hormone"

to intermediaries with androgenic activity. A relative deficiency of non-androgenic hormones might result thereby.

The postulation has also been advanced that a major factor involved in controlling the circulating levels of adrenal cortical hormones may be the rate of their metabolism by peripheral tissue (41). If the rate of destruction of 17-hydroxycorticosteroids by peripheral tissue were exceptionally rapid the hormone pattern observed in these patients could result. The failure of 17-hydroxycorticosteroid blood levels to decrease rapidly after the withdrawal of cortisone therapy in the children in this study makes this interpretation unlikely.

In patients with congenital adrenal hyperplasia there are two primary manifestations which demand therapy. These are early virilization and a stunting of eventual growth because of early epiphyseal closure. Until the introduction of cortisone therapy these demands had not been met adequately.

It would seem that cortisone therapy is accomplishing its desired result in the control of both of these problems. The observations in the present study are in conformity with those of others who have reported (3) clinical and chemical evidence that virilization is being controlled by cortisone therapy. Chemically they have shown uniformly a striking reduction in the urinary 17-ketosteroid excretion levels; clinically, although there has been no regression in the abnormalities originally present in the voice, size of the phallus, and amount and distribution of sexual hair, there have been more subtle indications of a decreasing masculinity. These consist of a more subdued behavior with less irritability, boisterousness and aggressiveness in all cases; subjectively decreased strength in two cases; and a marked improvement in the symptoms of priapism in the one case in which this had been a problem.

Therapy has not been in progress long enough to allow final evaluation of effects on somatic growth. However, suggestive evidence bearing on this point is furnished by the growth data on Patient 3. Her growth of 6 inches during eighteen months of cortisone therapy, without any further skeletal maturation, considerably improves her prognosis for ultimately attaining satisfactory growth.

A third problem with serious implications in these children is the psychologic disturbance resultant from their peculiar physical, sexual and social status. One of the most important results of any therapy would be the minimizing of this psychologic trauma. Several quite different situations may exist, depending chiefly upon the age of the individual patient at the time diagnosis is established. The need for decision regarding possible benefits of therapy and preferable sex role becomes, quite apparently, an individual matter. In the female pseudohermaphrodite who has been

raised as a girl and who comes under therapy before excessive virilization has occurred, cortisone offers an opportunity to prevent or minimize the features of masculinization, to attain normal growth and to develop a more psychologically stable sexual role. Plastic repair of the external genitalia may greatly contribute to the overall goal. The female pseudohermaphrodite raised as a girl, who is of an age at which these abnormalities are irreversible, may respond to cortisone therapy with breast development plus the establishment of menstrual cycles with estrogenizing changes in the vaginal mucosa (3). The female pseudohermaphrodite who has been raised as a boy may also be treated with cortisone, but less satisfactorily. If the child is young enough, however, therapy may allow greater realization of growth potential. Surgical extirpation of female internal genitalia in addition may remove possible postpubertal feminizing changes.

Males with congenital adrenal hyperplasia (macrogenitosomia praecox) may also benefit from cortisone if therapy is instituted early enough. In such cases the opportunity may be given for more satisfactory somatic growth before epiphyseal closure than otherwise would be expected. The evidences of early virilization also may be minimized, allowing more normal social relationships. In the case of the male in whom the diagnosis has been made or treatment started for the first time at an age at which skeletal maturation has taken place, cortisone therapy probably has little to offer.

The possibility of serious side effects from prolonged cortisone therapy exists and merits careful consideration. Although no serious side effects have been noted in the patients presented here, minor changes have been observed in 3 of the 4. These have consisted of slightly increased pigmentation, particularly of scar areas, in 1 patient; and rapid weight gain and "moon facies" in 2 patients. It is of special interest that in these latter patients evidence of side effects disappeared with the reduction of cortisone dosage, without the sacrifice of therapeutic efficacy. Because of the incompletely evaluated danger of side effects of cortisone therapy, careful clinical and laboratory observations should be made on patients so treated.

SUMMARY

1. The results of metabolic studies and clinical observations in 4 patients with congenital adrenal hyperplasia are presented.

2. Circulating blood levels of 17-hydroxycorticosteroids before, during and after cortisone therapy are reported.

3. Mention is made of preliminary data regarding the circulating blood levels of ACTH.

4. Evidence is presented which is interpreted as supporting the rationale for prolonged cortisone therapy in patients with congenital adrenal hyperplasia.

5. Various possible mechanisms of pathogenesis of this syndrome are discussed in relation to new data presented.

REFERENCES

1. WILKINS, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Springfield, Illinois, Charles C Thomas, Publisher, 1950.
2. WILKINS, L.: Hyperadrenocorticism, *Pediatrics* **3:** 533, 1949.
3. WILKINS, L.; LEWIS, R. A.; KLEIN, R.; GARDNER, L. I.; CRIGLER, J. F., JR.; ROSEMBERG, E., and MIGEON, C. J.: Treatment of congenital adrenal hyperplasia with cortisone, *J. Clin. Endocrinol.* **11:** 1, 1951.
4. HINMAN, F., JR.: Sexual trends in female pseudohermaphrodism, *J. Clin. Endocrinol.* **11:** 477, 1951.
5. WILKINS, L.; LEWIS, R. A.; KLEIN, R., and ROSEMBERG, E.: The suppression of androgen secretion by cortisone in a case of congenital adrenal hyperplasia, *Bull. Johns Hopkins Hosp.* **86:** 249, 1950.
6. WILKINS, L.; LEWIS, R. A.; KLEIN, R., and ROSEMBERG, E.: Die Wirkung von Cortisone auf die Ausscheidung der 17-Ketosteroids und anderer Steroide bei Patienten mit kongenitaler Niebennierenhyperplasie, *Helvet. paediat. acta* **5:** 418, 1950.

7a. CALLOW, N. H.; CALLOW, R. K., and EMMENS, C. W.: Colorimetric determination of substances containing grouping—CH_2CO—in urine extracts as indication of androgen content, *Biochem. J.* **32:** 1312, 1938.

b. CAHEN, R. L., and SALTER, W. T.: Urinary 17-ketosteroids in metabolism. I. Standardized chemical estimation, *J. Biol. Chem.*, **152:** 489, 1944.

8. PETERS, J. P., and VAN SLYKE, D. D.: Quantitative Clinical Chemistry. Vol. II. Methods. Baltimore, Williams and Wilkins Company, 1932, p. 829.
9. VAN SLYKE, D. D., and NEILL, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I., *J. Biol. Chem.* **61:** 523, 1924.
10. RANDOLPH, T. G.: Blood studies in allergy. I. The direct counting chamber determination of eosinophils in propylene glycol-aqueous stains, *J. Allergy* **15:** 89, 1944.
11. NELSON, N.: Photometric adaptation of Somogyi method for determination of glucose, *J. Biol. Chem.* **153:** 375, 1944.
12. NELSON, D. H., and SAMUELS, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood; 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12:** 519, 1952.
13. MASON, A. S.: Adrenal virilism: abnormal response to ACTH, *Proc. Roy. Soc. Med.* **44:** 162, 1951.
14. LEWIS, R. A.; ROSEMBERG, E., and WILKINS, L.: The effects of 17-hydroxy-11-dehydrocorticosterone upon the adrenals of normal and of hypophysectomized rats maintained with adrenocorticotropin, *Endocrinology* **47:** 414, 1950.
15. BARTTER, F. C.: ALBRIGHT, F.; FORBES, A. P.; LEAF, A.; DEMPSEY, E., and CARROLL, E.: The effects of adrenocorticotropic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia: an attempt to explain and correct its disordered hormonal pattern, *J. Clin. Investigation* **30:** 237, 1951.
16. LEWIS, R. A., and WILKINS, L.: The effect of adrenocorticotrophic hormone in congenital adrenal hyperplasia with virilism and in Cushing's syndrome treated with methyl testosterone, *J. Clin. Investigation* **28:** 394, 1949.
17. VENNING, E. H.; KAZMIN, V. E.; RIPSTEIN, M.; MCALPINE, H. T., and HOFFMAN,

M. M.: The effect of adrenocorticotropin on the excretion of adrenal metabolites in normal human subjects, *J. Clin. Endocrinol.* **10:** 583, 1950.

18. Bliss, E. L.; Rubin, S., and Gilbert, T.: The effect of adrenalin on adrenal cortical function, *J. Clin. Endocrinol.* **11:** 46, 1951.
19. Sydnor, K. L.; Kelley, V. C.; Raile, R. B.; Ely, R. S., and Sayers, G.: Unpublished data.
20. Sydnor, K. L., and Sayers, G.: A technic for determination of adrenocorticotrophin in blood, *Proc. Soc. Exper. Biol. & Med.* **79:** 432, 1952.
21. Sayers, G., and Sayers, M. A.: Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress, *Endocrinology* **40:** 265, 1947.
22. Sayers, G.: Regulation of the secretory activity of the adrenal cortex, *Am. J. Med.* **10:** 539, 1951.
23. Recant, L.; Hume, D. M.; Forsham, P. H., and Thorn, G. W.: Studies on the effect of epinephrine on the pituitary-adrenocortical system, *J. Clin. Endocrinol.* **10:** 187, 1950.
24. White, F. P., and Sutton, L. E., Jr.: Observations on pituitary-adrenal response to epinephrine, *Pediatrics* **5:** 876, 1950.
25. White, F. P., and Sutton, L. E.: Adrenogenital syndrome with associated episodes of hypoglycemia, *J. Clin. Endocrinol.* **11:** 1395, 1951.
26. Kepler, E. J., and Locke, W.: The adrenals. I. Chronic adrenal hyperfunction, *in* Williams, R. H.: Textbook of Endocrinology. Philadelphia, W. B. Saunders Co., 1950, pp. 180–248.
27. Wilkins, L.; Klein, R., and Lewis, R. A.: The response to ACTH in various types of adrenal hyperplasia, *in* Proceedings of the First Clinical ACTH Conference, edited by J. R. Mote. Philadelphia, The Blakiston Co., 1950, p. 184.
28. Shepard, T. H., II, and Clausen, S. W.: Case of adrenogenital syndrome with hypertension treated with cortisone, *Pediatrics* **8:** 805, 1951.
29. Panos, T. C.: Female pseudohermaphroditism with hypoadrenia, *Pediatrics* **5:** 972 1950.
30. Zuelzer, W. W., and Blum, A., Jr.: Adreno-cortical insufficiency in infants with the adrenogenital syndrome, *J. Pediat.* **35:** 344, 1949.
31. Barnett, H. L., and McNamara, H.: Electrolyte balances in male infant with adrenocortical insufficiency and virilism. Effect of desoxycorticosterone acetate and salt therapy with special reference to potassium, *J. Clin. Investigation* **28:** 1498, 1949.
32. Harris, C. F., and Scowen, E. F.: Foetal adrenal hyperplasia, *Arch. Dis. Child.* **26:** 423, 1951.
33. Carlgren, L. E.: Congenital hyperplasia of the adrenals with adrenal cortical insufficiency, *Acta paediat.* **38:** 71, 1949.
34. Talbot, N. B.; Zygmuntowicz, A. S.; Wood, M., and Christo, E.: Observations on adrenal cortical "sugar-fat-nitrogen" hormone ("11-17-OCS") and "17-ketosteroid precursor" production by normal and abnormal individuals of various ages with comments on the fact that (a) there may be two ACTH's and (b) the normal adrenal cortex may not produce true androgens, *in* Proceedings of the First Clinical ACTH Conference, edited by J. R. Mote. Philadelphia, The Blakiston Co. 1950, p. 32.
35. Talbot, N. B.; Wood, M. S.; Campbell, A. M.; Christo, E., and Zygmuntowicz, A. S.: Concerning the probability that there are at least two adrenocorticotrophic hormones in the human being, *in* Proceedings of the Second Clinical ACTH Conference, edited by J. R. Mote. Philadelphia, The Blakiston Co., 1951, vol. 1, p. 20.

36. Conn, J. W.; Louis, L. H., and Fajans, S. S.: The probability that compound F (17-hydroxycorticosterone) is the hormone produced by the normal human adrenal cortex, *Science* **113:** 713, 1951.
37. Nelson, D. H.; Reich, H., and Samuels, L. T.: Isolation of a steroid hormone from the adrenal-vein blood of dogs, *Science* **111:** 578, 1950.
38. Haines, W. J.; Johnson, R. H.; Brunner, M. P.; Pabst, M. L., and Kuizenga, M. H.: Study of the carbohydrate-regulating hormone, *Fed. Proc.* **8:** 203, 1949.
39. Pincus, G.; Hechter, O., and Zaffaroni, A.: The effect of ACTH upon steroidogenesis by the isolated perfused adrenal gland, *in* Proceedings of the Second Clinical ACTH Conference, edited by J. R. Mote. Philadelphia, The Blakiston Co., 1951, vol. 1, p. 40.
40. Lloyd, C. W.; Lobotsky, J.; Jones, J.; Fredericks, J., and Wyatt, T. C.: Hormone studies in a case of adrenogenitalism due to neoplasm of the adrenal cortex, *J. Clin. Endocrinol.* **11:** 857, 1951.
41. Sayers, G.: The adrenal cortex and homeostasis, *Physiol. Rev.* **30:** 241, 1950.
42. Jackson, R. L., and Kelly, H. G.: Growth charts for use in pediatric practice, *J. Pediat.* **27:** 215, 1945.

MEASUREMENT OF THE EXCRETION OF CORTISONE AND COMPOUND F, USING THE PORTER-SILBER METHOD

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THE excretion in the urine of adrenal cortical steroids and their metabolites has served as an index of adrenal cortical activity. Of the many biologic and chemical methods applied to the measurement of corticoid excretion, the recent one of Porter and Silber (1) appeared to us to possess the advantages of simplicity of performance and of specificity for certain 17-hydroxycorticoids. Both cortisone and compound F are included in the 17,21-dihydroxy-20-ketosteroid group of compounds measured by this technique.

A study of urinary excretion of steroids of patients receiving cortisone, compound F, and corticotropin forms the basis of this report. In our hands the Porter-Silber method was not reliable in measuring steroid concentrations in plasma or feces.

METHOD

The original Porter-Silber method of measuring steroids was modified slightly to permit analysis of several specimens simultaneously. Urine was collected over a measured period of time, with a small amount of chloroform added as a preservative. The specimens were stored in the refrigerator and analyzed within five days. A 20-ml. aliquot of urine was extracted with 20 ml. of chloroform. The chloroform was separated by centrifugation. A 4-ml. portion of chloroform extract was placed in each of two 100×25-mm. test tubes and evaporated in a vacuum oven at 40° C and at a pressure of 28–30 mm. of Hg. To each of the tubes containing the dry steroid residue was added 1 ml. of methanol and the reaction was then completed as described by Porter and Silber, using one tube as the specimen blank. Standard solutions of cortisone in methanol were used for reference.

This method resulted in recovery of 90 per cent ± 3 per cent of 5γ of cortisone added to 20 ml. of urine.

Because of lack of chromatographic or chemical proof that the chromogenic material extracted was only 17,21-hydroxysteroid, the term "corticoid" will be used in referring to the Porter-Silber chromogen measured by the method employed.

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Using the procedure described, no steroid was demonstrable in urine from normal people. Carroll, McAlpine and Noble (2) report normal "steroid" excretory rates equivalent to 0.1 to 0.4 mg. per twenty-four hours in 8 adult males and 0 to 0.86 mg. per twenty-four hours in 7 adult females. In using 25-ml. aliquots of urine per test and serial extractions of the urine with chloroform, they may have effected a more complete extraction of Porter-Silber chromogen from the urine than we did.

TABLE 1

Diagnosis	Number of patients
Rheumatoid arthritis	7
Gout	1
Addison's disease	3
Normal control	3

SUBJECTS AND PROCEDURE

Fourteen males received cortisone by mouth (Table 1). One patient received a single dose of 25 mg. of cortisone orally (Fig. 1). The others received cortisone, 50 mg. every six hours, for four doses (200 mg.) (Fig. 2).

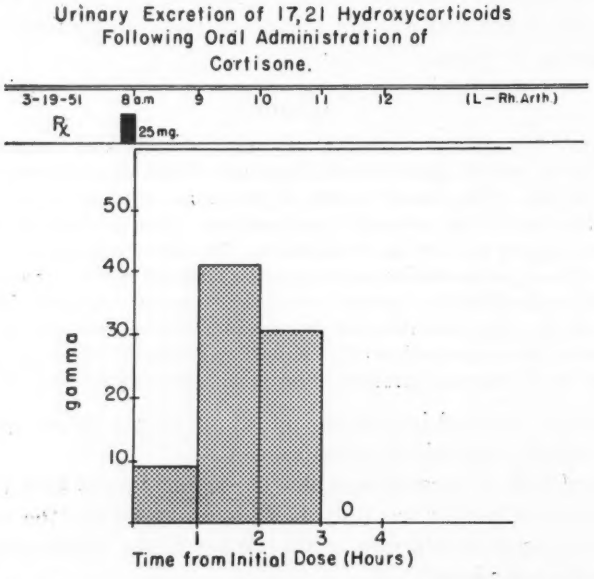

FIG. 1. Excretion of "corticoid" following a single oral dose of cortisone acetate, 25 mg. Diagnosis: rheumatoid arthritis.

One patient was given 100 mg. every six hours for four doses (400 mg.) for a second test. The normal controls received cortisone, 50 mg. every six hours for three doses and a fourth dose twelve hours after the third (Fig. 3). In addition, 3 patients with rheumatoid arthritis received cortisone intramuscularly, 50 mg. every six hours for four doses. A control specimen of urine was collected prior to giving cortisone, and all urine passed for the

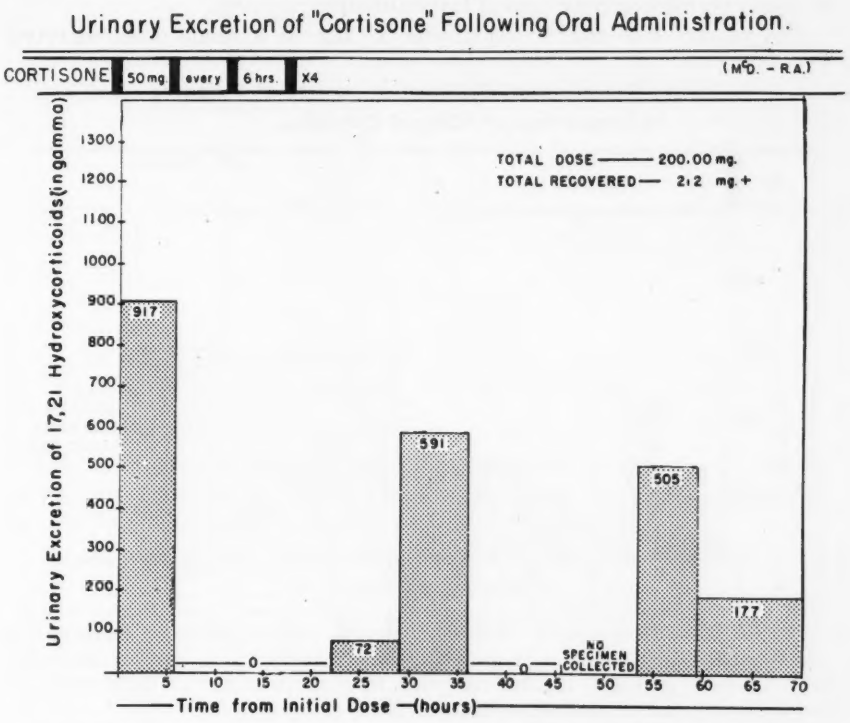

FIG. 2. Excretion of "corticoid" during and after oral administration of cortisone acetate, 50 mg. every 6 hrs. ×4. Note irregular rate of excretion. Diagnosis: rheumatoid arthritis.

ensuing forty-eight to ninety hours was collected. Urine collections were made at intervals of fifteen minutes to one hour after the initial dose and at longer periods thereafter. None of the patients had renal disease or renal insufficiency as determined by urinalysis and blood urea determinations.

In this series it is apparent from the excretion charts (Fig. 2A) that within fifteen to twenty minutes after ingestion of 50 mg. of cortisone, measurable quantities of "corticoid" appear in the urine. It is of interest to note

that usually after a given dose of cortisone there is an initial high excretion rate which gradually declines. Occasionally during the period following a single dose of cortisone a collection of urine free of "corticoid" is obtained between two collections containing "corticoid." No explanation for this fluctuation in excretion rate following a given dose of cortisone is possible at this time, although a comparison with the data of Carroll *et al.* (2) indicates that their method of extraction might have increased the sensitivity of assay, permitting detection of trace amounts excreted.

The patient who received cortisone, 25 mg. as a single dose, excreted

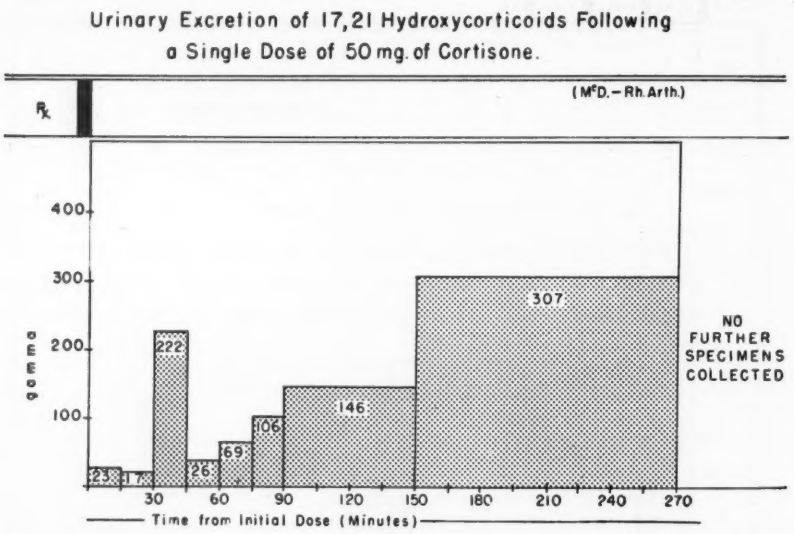

FIG. 2A. Rate of excretion of "corticoid" following a single oral dose of cortisone acetate, 50 mg. Urine collections were made every fifteen minutes for ninety minutes and at longer intervals thereafter. Control urine prior to administration of cortisone contained no "corticoid." Same patient as in Figure 2.

0.082 mg. of "corticoid" in the ensuing four hours (Fig. 1). In the others, recovery ranged from 0.5 to 4.6 mg. following a total dose of 200 mg. of cortisone orally (50 mg. every six hours for four doses) (Fig. 2). One patient excreted no "corticoid" while receiving cortisone, 50 mg. orally every six hours for four doses. When the dose was increased to 100 mg. every six hours, he excreted quantities of "corticoid" ordinarily excreted by others after 50 mg. doses of cortisone. One patient (Fig. 7), who had been on a maintenance dose of 25 mg. of cortisone three times daily for six months, excreted no measurable quantities of "corticoid" in 24-hour urine collections until ACTH was given intravenously. The 3 patients with Addison's

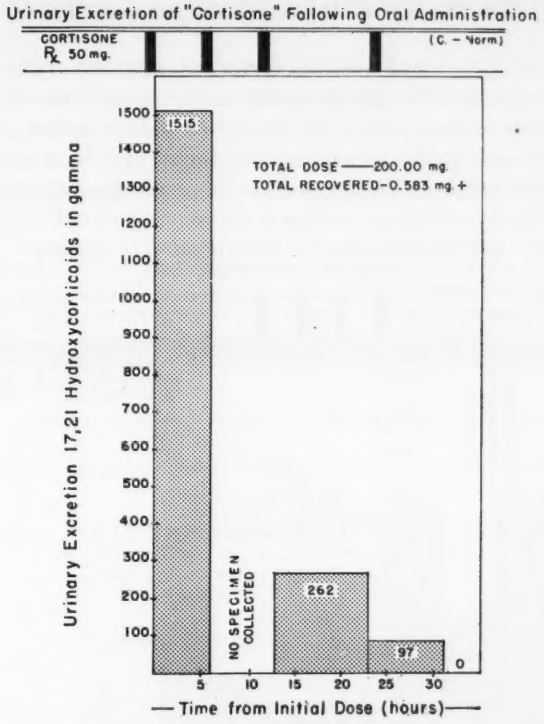

FIG. 3. Excretion of "corticoid" during and after oral administration of cortisone acetate, 50 mg. every 6 hrs. ×3, with a fourth dose 12 hours after the third. Note irregular rate of excretion. Normal male.

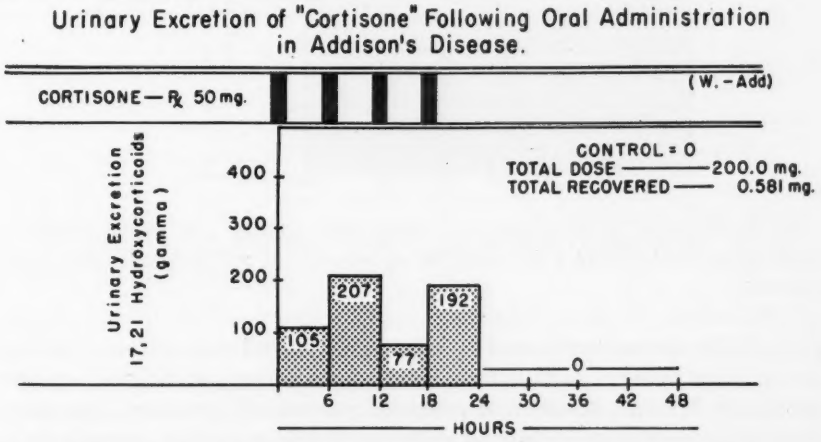

FIG. 4. Excretion of "corticoid" during and after oral administration of cortisone acetate, 50 mg. every 6 hrs. ×4. That cessation of excretion followed abruptly upon cessation of administration of cortisone is interesting but not significant, for excretion by other patients as well as normal subjects was often similar. Diagnosis: Addison's disease.

disease (Fig. 4), given oral doses of cortisone, excreted "corticoid" just as the normal males and the patients with rheumatoid arthritis, except that in every case excretion ceased within six to twelve hours after stopping cortisone. This cannot be interpreted in terms of "demand" or "utilization" by patients with Addison's disease, since similar patterns of excretion

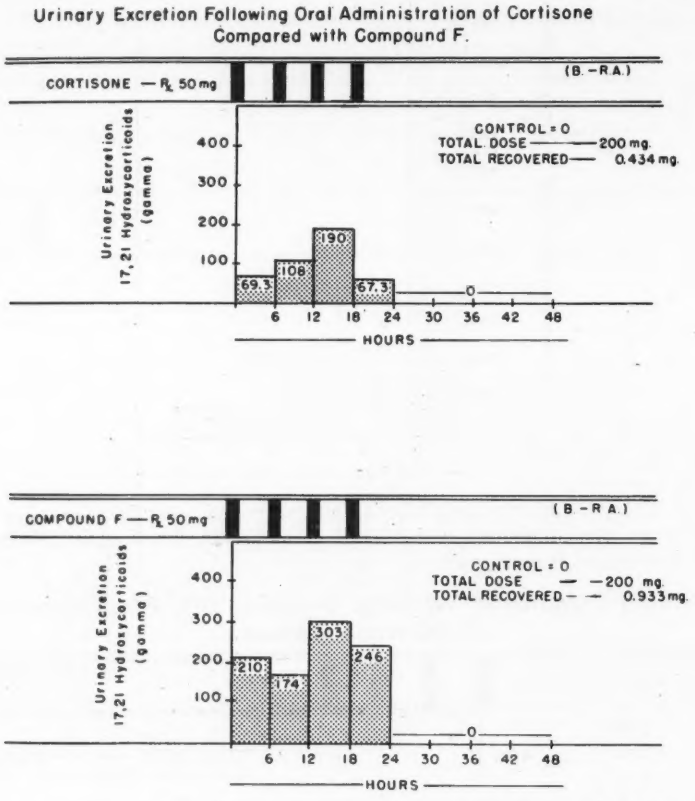

FIG. 5. Comparison of excretion of "corticoid" following oral administration of cortisone and of compound F in doses of 50 mg. every 6 hrs. ×4. Diagnosis: rheumatoid arthritis.

were seen in normal males and in some patients with rheumatoid arthritis. One of these patients had bilateral total adrenalectomy for adrenal cortical carcinoma. The neoplasms had produced no cortical hormones, and three years after surgery there was no evidence of presence of any neoplastic or normal adrenal tissue.

In 3 patients who received intramuscular administration of cortisone, 50

mg. every six hours for four doses, no "corticoid" was found in the urine.

Our data indicate that the rate of excretion of cortisone varies markedly for different individuals. These data are not adequate to define the relationship between dose and amount excreted by any one person.

There was no difference in amount of steroid excreted by ill subjects and normal controls. Because the total amounts excreted are so small, it is

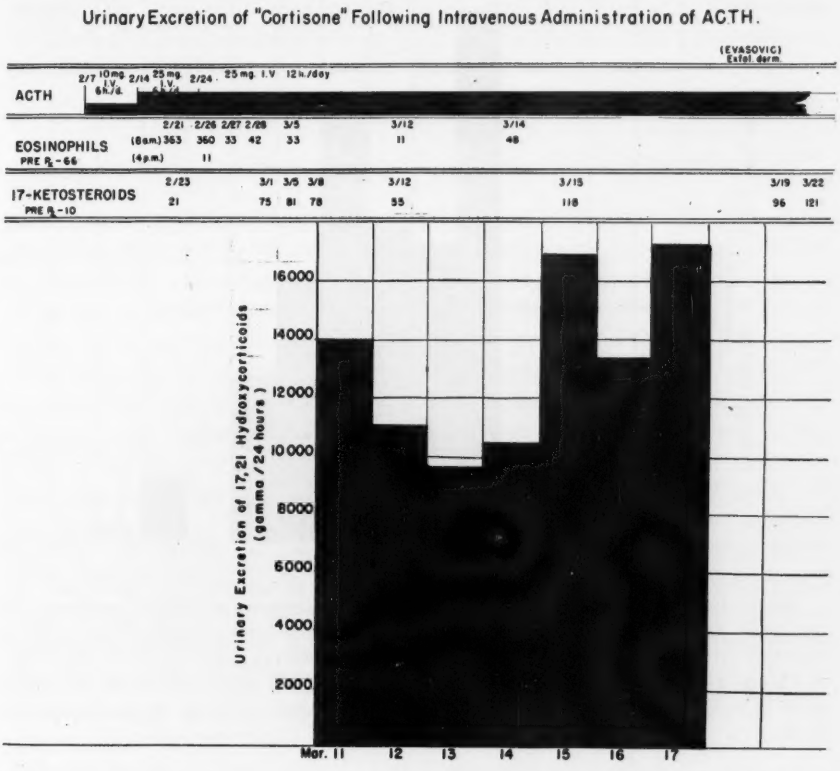

FIG. 6. Excretion of "corticoid" and 17-ketosteroid by a patient receiving large doses of corticotropin intravenously. This is the largest amount excreted by any patient in our series. Diagnosis: psoriatic arthritis.

unlikely that it will be possible to ascertain any significant differences in the requirement or utilization of cortisone by comparing ill and normal subjects.

Two patients from the cortisone group were given compound F, 50 mg. orally every six hours for four doses (200 mg.). Urine collections were closed at the end of each six-hour period following a dose of compound F, and six-hour collections were continued for an additional 24-hour period.

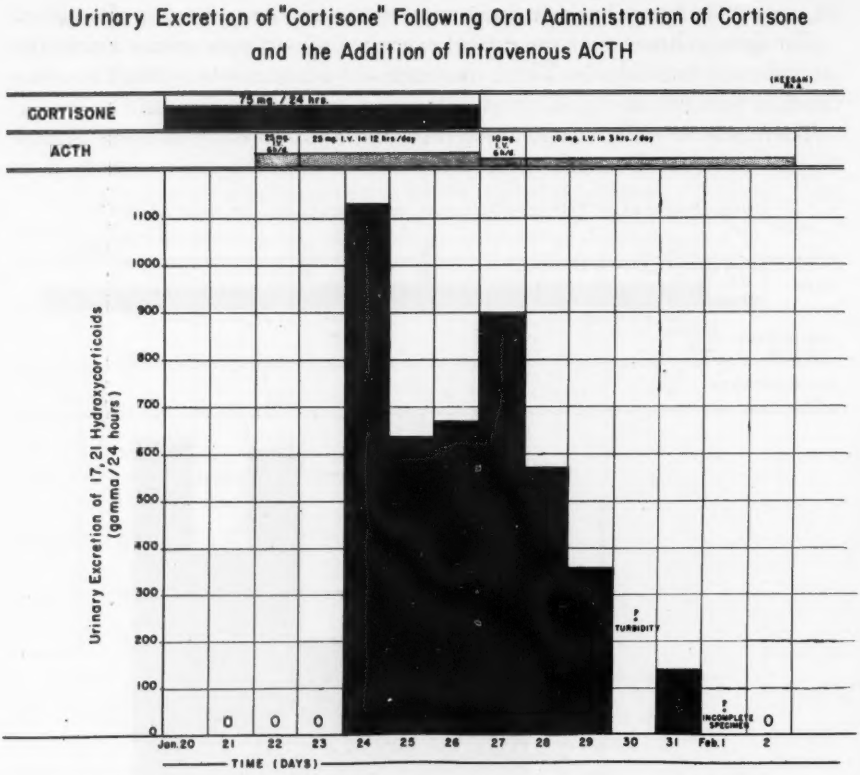

FIG. 7. Excretion of "corticoid" by a patient on maintenance doses of cortisone, 25 mg. t.i.d., before and after the addition of corticotropin intravenously. Note drop in excretion of "corticoid" upon stopping cortisone and reducing daily dose of corticotropin to 10 mg. given intravenously over a 3-hour period. This small dose of corticotropin given in only 3 hours was adequate to maintain a clinical remission of rheumatoid arthritis.

Quantities of "corticoid" excreted following administration of compound F were comparable to those following administration of cortisone (Fig. 5).

A separate group of male patients received corticotropin in daily doses of 25 mg. in 1,000 ml. of 5 per cent glucose in water given intravenously over a twelve- to sixteen-hour period. Urinary excretion rates of "corticoids" were measured at varying times before and during treatment (Figs. 6 and 7).

Those receiving corticotropin intravenously excreted large amounts of cortisone-like steroid in the urine. One patient with generalized exfoliative psoriasis, in the sixth week of treatment, when receiving a daily dose of

25 mg. of ACTH by 12-hour intravenous drip, excreted 10 to 17 mg. of "corticoids" daily. The 17-ketosteroid excretion reached 118 mg. per day at this time (Fig. 6). Other patients responded similarly to the stimulus of ACTH. The patient (Fig. 7) who received 75 mg. of cortisone daily (25 mg. t.i.d., orally) and excreted no measurable quantity of "corticoids," was given ACTH (25 mg. intravenously daily) with no change in cortisone dosage. No "corticoid" was excreted until after the third day, when excretion rose to 1.1 mg. in twenty-four hours, diminishing slowly upon stopping cortisone and reducing the dose of ACTH to 10 mg. daily.

SUMMARY

It is possible to measure the concentration of 17,21-hydroxy-20-ketosteroids ("corticoid") in urine with sufficient accuracy to detect changes in excretion. With the technique employed, normal individuals did not excrete steroid in measurable quantities. The oral administration of cortisone and compound F in 25-mg. to 50-mg. doses resulted in excretion of small quantities of "corticoid"; total excretion following 200 mg. of cortisone (50 mg. every six hours) was less than 5 mg. Excretion of "corticoid" was rapid, often being greatest after the first dose; it appeared in the urine within fifteen minutes after the initial dose of cortisone or compound F and was usually no longer present in measurable quantities forty-eight to seventy-two hours after therapy was stopped. No "corticoid" was found in the urine following intramuscular administration of 50 mg. of cortisone every six hours for a total of 200 mg. in twenty-four hours. Following oral administration of cortisone or compound F, 50 mg. every six hours for four doses, no significant differences in rates of excretion were noted between normal males and patients suffering from rheumatoid arthritis, from gout, or from Addison's disease. There was, however, some variation in the rate of excretion by the individual test subjects. Following administration of ACTH, the production by the adrenal cortex of corticosteroids resulted in the excretion of large quantities of 17,21-hydroxy-20-ketosteroids as well as of 17-ketosteroids.

REFERENCES

1. Porter, Curt, C., and Silber, Robert H.; Quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201 (July) 1950.
2. Carroll, K. K.; McAlpine, H. T., and Noble, R. L.: Studies on the excretion of cortisone and compound F using the Porter-Silber method, *Canad. M.A.J.* **65**: 363 (Oct.) 1951.

THE EFFECT OF ANDROGENS ON THE URINARY EXCRETION OF KETOSTEROIDS, NONKETONIC ALCOHOLS AND ESTROGENS*†

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INTRODUCTION

THE development of a method for the colorimetric estimation of urinary steroid alcohols (3) and a fluorometric method for the estimation of urinary estrogens (2) makes it possible to carry out simultaneous studies of these components as well as ketosteroids in the urine of patients under various types of hormone therapy. This report will be concerned with the effect of the administration of dehydroepiandrosterone[1] and testosterone on the urinary excretion of ketosteroids, steroid alcohols and fluorogenic phenols[2] (FP) in a series of 6 patients with cancer of the breast (female), 1 patient with cancer of the prostate gland and 4 patients with rheumatoid arthritis.[3]

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Preliminary reports of some of these data were presented at the V International Cancer Congress, Paris, July 16–22, 1950; at a conference on "Metabolic Breakdown of Steroids" held at the Ciba Foundation, London, England, August 9–11, 1950; and at a symposium on "Steroids in Experimental and Clinical Practice" at Cuernavaca, Mexico, January 15–18, 1951 (1).

† The authors are grateful to Ciba Pharmaceutical Products, Inc. for their generosity in supplying the hormones used in these studies.

[1] The nomenclature in this paper will conform with that proposed by Fieser and Fieser (2).

[2] The term "fluorogenic phenols" (FP) refers to the material yielding fluorescence in the sulfuric acid reaction and includes the three major estrogens when they are present.

[3] We are indebted to Dr. Walter Bauer and Dr. William S. Clark for making these specimens available to us.

METHODS

Twenty-four-hour urine specimens were collected during preliminary control periods, periods of therapy and post-treatment control periods. Daily determinations of creatinine were performed to check completeness of the collections.

An aliquot of each 24-hour specimen was hydrolyzed by boiling for ten minutes with 15 volumes per cent of concentrated hydrochloric acid and the neutral fraction isolated in the usual manner.

Ketosteroids were measured by the Nathanson-Wilson modification of the Holtorff-Koch method (4, 5) using dehydroepiandrosterone as the standard. The results were calculated both as milligrams and as microequivalents[4] of dehydroepiandrosterone per twenty-four hours.

Total neutral steroid alcohols were measured by the dinitrophthalate method (3) and the results expressed as μEq. of total alcohols per twenty-four hours. Nonketonic alcohols were computed as the difference between total alcohols (μEq.) and ketosteroids (μEq.). It is assumed as a first approximation that the average ketosteroid contains one hydroxyl and one carbonyl group.

The processing of the urine for phenols, and the fluorometric analysis have been described (6). When sufficient material was available the phenolic fraction was separated by counter-current distribution and the individual estrogens analyzed and characterized.

RESULTS

Chemical findings: The steroid excretion patterns obtained in 10 patients are summarized in Table 1. All 10 patients received testosterone and, of these, 3 received dehydroepiandrosterone prior to the testosterone.

Ketosteroids: In all patients the urinary ketosteroid excretion rose to values ranging from twice to thirteen times the normal level. This was true both in the testosterone and the dehydroepiandrosterone experiments.

Nonketonic alcohols: The pattern of response of the nonketonic steroid alcohols was irregular after the administration of either testosterone or dehydroepiandrosterone. Under testosterone therapy, 2 (R.MacD. and F. R.) of the 5 patients with cancer of the breast showed an increased excretion of nonketonic steroid alcohols. In the remaining 3 (R.C., M.C. and J.W.), markedly reduced amounts of nonketonic alcohols were found. The patient with cancer of the prostate gland excreted increased amounts of nonketonic alcohols during testosterone therapy. Three (E.A., J.H. and S.K.) of the 4 patients with rheumatoid arthritis who were treated with testosterone showed a marked fall in nonketonic alcohol excretion, but the fourth (E.J.B.) showed no significant change.

Both breast cancer patients (R.C. and R.MacD.) who received dehydroepiandrosterone excreted increased amounts of nonketonic alcohols but the arthritic patient (E.A.) showed a decreased excretion of this complex when the same hormone was administered.

[4] Microequivalent is defined as follows: μEq. = mol. wt. $\times 10^{-6}$/number of hydroxyl or ketone groups.

TABLE 1. THE EFFECT OF ANDROGENS ON THE URINARY EXCRETION OF KETOSTEROIDS, NONKETONIC STEROID ALCOHOLS AND ESTROGENS

Patient	Age (yrs.)	Sex	Therapy	KS*	NKA**	FP†	Clinical effects
				per cent change from control level			
Rheumatoid arthritis							
E. A.	68	F	DHA‡	+ 238	−52	− 9	None
E. A.	68	F	TP§	+ 246	−18	− 8	None
E. J. B.	39	M	TP	+ 687	− 3	+17	None
J. H.	48	M	TP	+ 454	−95	− 3	None
S. K.	59	M	TP	+1321	−84	+38	None
Breast cancer							
R. C.	69	F	DHA	+ 266	+17	+44	None
R. C.	69	F	TP	+ 454	−62	+61	None; increased pain
M. C.	55	F	TP	+ 326	−41	−10	Improvement
R. MacD.	35	F	DHA	+ 184	+84	+32	None
R. MacD.	35	F	TP	+ 401	+47	+53	Slight relief of pain
F. R.	57	F	TP	+ 290	+63	+97	Deterioration; improvement later
J. W.	64	F	TP	+ 790	−41	+81	Deterioration
Prostatic cancer							
J. S.	59	M	TP	+ 260	+29	+23	Increased pain; elevation of acid phosphatase

* Ketosteroids.
** Nonketonic alcohols.
† Fluorogenic phenols.
‡ Dehydroepiandrosterone acetate.
§ Testosterone propionate.

FP: These assays were carried out in all 10 patients. Of the 6 cancer patients who received testosterone, 5 showed an increased FP excretion and 1 showed no significant change (M.C.). A significant increase in FP excretion was observed in both patients with cancer of the breast who received dehydroepiandrosterone. During testosterone therapy, 2 of 3 arthritic patients had a significantly higher FP excretion, but another patient with rheumatoid arthritis who received both hormones showed no change.

Detailed observations: The daily studies which were carried out in the cases of R.C. (female, age 69, cancer of the breast), R.MacD. (female, age

35, cancer of the breast, castrated by x-ray) and E.A. (female, age 68, rheumatoid arthritis) are charted in Figures 1–9. In all 3 patients the administration of either dehydroepiandrosterone or testosterone was followed by a striking rise in the excretion of both ketosteroids and *total* steroid alcohols (Figs. 1, 2, 3) and a fall to or below the control levels when the administration of the hormone was terminated.

When the daily nonketonic alcohol excretion of these 3 patients is plotted

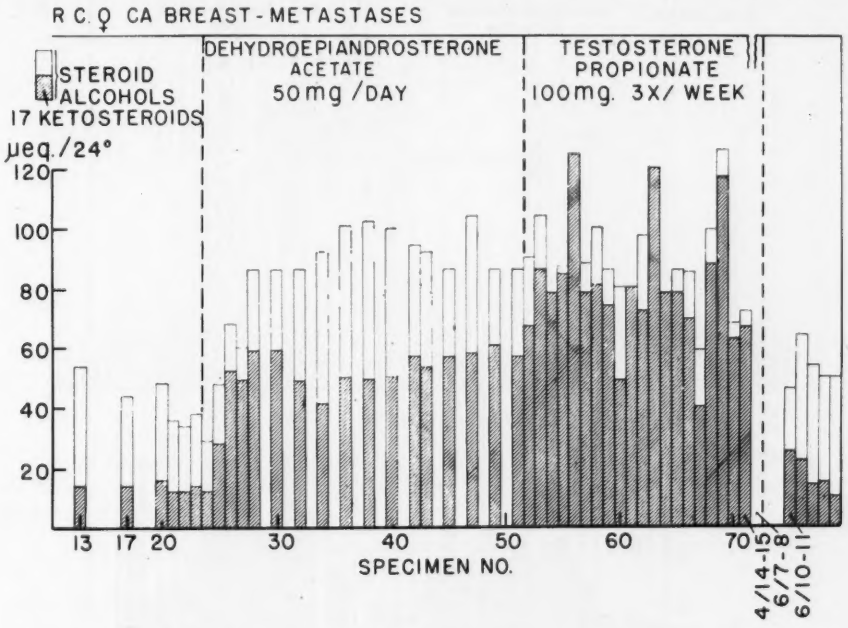

FIG. 1. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of ketosteroids and steroid alcohols in a 69-year-old woman with metastatic carcinoma of the breast.

in terms of percentage change from the pre-treatment control level, variations in excretion patterns become apparent. In Patient R.C. (Fig. 4), during the period of dehydroepiandrosterone administration there was an initial fall in nonketonic alcohol excretion, followed by an increased excretion of this complex. When testosterone was substituted for dehydroepiandrosterone, the nonketonic alcohols promptly fell to levels significantly below those observed in the control period. This picture may be contrasted with that shown by Patient R.MacD. (Fig. 5), in whom the administration of both dehydroepiandrosterone and testosterone caused an increased excretion of nonketonic steroid alcohols. A further variation was shown by Patient E.A. (Fig. 6), in whom the nonketonic alcohol excretion was markedly depressed both by dehydroepiandrosterone and by testosterone.

The daily FP excretion was followed in the same 3 patients (Figs. 7, 8, 9). Although there were some day-to-day variations, the average daily excretion during the periods of dehydroepiandrosterone administration was significantly higher than during the control periods in the 2 cancer patients and was unchanged in the patient with rheumatoid arthritis. Testosterone

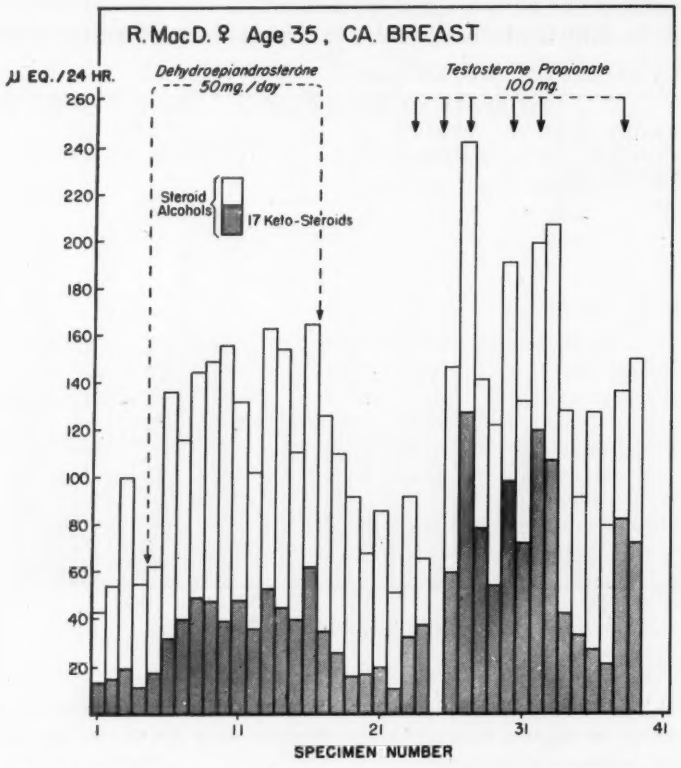

FIG. 2. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of ketosteroids and steroid alcohols in a 35-year-old woman with cancer of the breast who had been castrated (x-ray) two months previously.

administration subsequent to dehydroepiandrosterone produced a further rise in FP excretion in the 2 cancer patients and no change in the patient with rheumatoid arthritis.

In the 2 cancer patients sufficient material was accumulated to characterize the estrogens by counter-current distribution (Table 2). During the control period Patient R.C. did not excrete detectable amounts of the three major urinary estrogens, estrone, estradiol-17β and estriol, although the average FP excretion was 51 μg. per day. When dehydroepiandrosterone

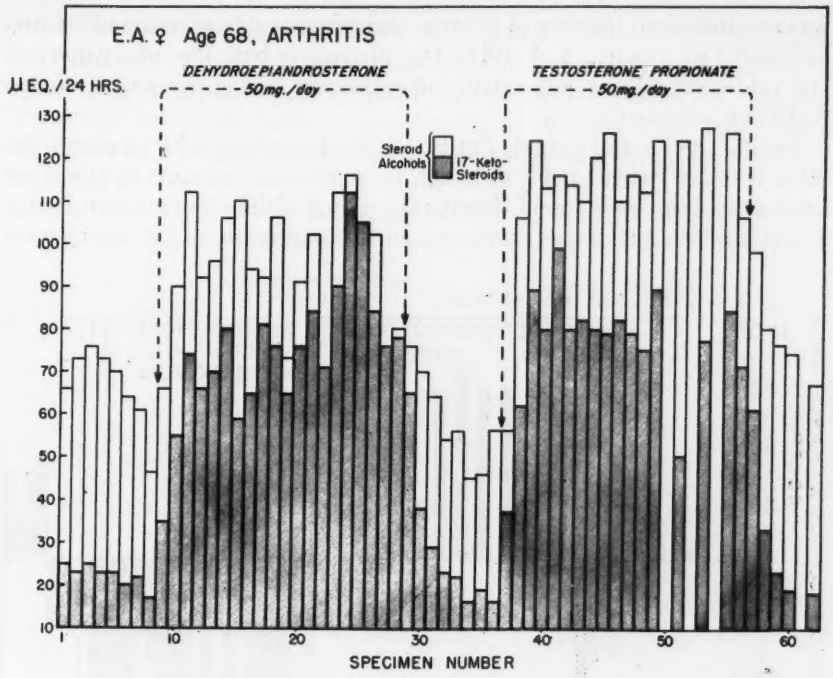

FIG. 3. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of ketosteroids and steroid alcohols in a 68-year-old woman with rheumatoid arthritis.

TABLE 2. THE EFFECT OF ANDROGENS ON THE URINARY EXCRETION OF ESTROGENS IN WOMEN

Patient	Age (yrs.)	Disease	Therapy	Estrogens (μgm./day)			
				Total*	E 1**	E 2†	E 3‡
R. C.	69	Ca. breast, met.	0	51	0	0	0
R. C.	69	Ca. breast, met.	DHA,# 50 mg./day	72	1.2	0	2.6
R. C.	69	Ca. breast, met.	TP,§ 100 mg. 3×week	82	2.4	<3	7.6
R. MacD.	35	Ca. breast, met.	0	47	<0.8	0	<0.9
R. MacD.	35	Ca. breast, met.	DHA, 50 mg./day	62	<1.0	<1.5	7
R. MacD.	35	Ca. breast, met.	TP, 100 mg. 3×week	72	2.2	3.3	12
E. A.	68	Rheumatoid arth.	0	80			
E. A.	68	Rheumatoid arth.	DHA, 50 mg./day	73			
E. A.	68	Rheumatoid arth.	TP, 50 mg./day	74			

* Total fluorogenic phenols as μgm. estrone/day.
** Estrone.
† Estradiol-17β.
‡ Estriol.
# Dehydroepiandrosterone acetate.
§ Testosterone propionate.

was administered, the titer of FP rose and recognizable amounts of estrone and estriol were excreted. A still further increase in both the total value and the values for estrone and estriol was observed during the period of testosterone administration.

During the control period, Patient R.MacD. excreted FP at about the same level as Patient R.C., although no significant amounts of the three known estrogens were found. During the period of dehydroepiandrosterone administration estriol was characterized, although estrone and estriol were

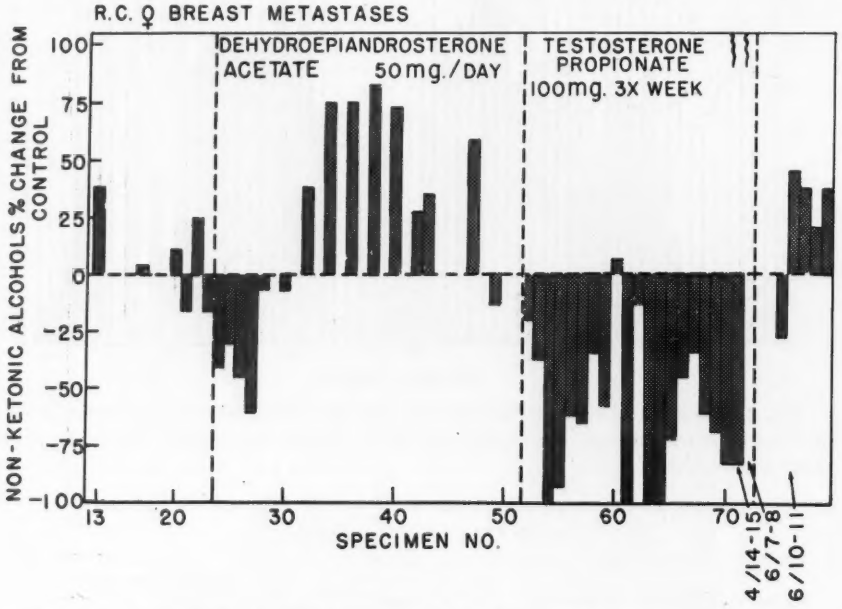

FIG. 4. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of nonketonic steroid alcohols in a 69-year-old woman with metastatic carcinoma of the breast.

not present in detectable amounts. However, when testosterone was given, all three compounds were excreted in significant amounts.

In Patient E.A. there was no significant change in FP excretion, hence no attempt was made to investigate the phenolic fraction by countercurrent distribution.

DISCUSSION

Clinical findings: The clinical effects of the administered hormones are indicated in the final column of Table 1. None of the patients with rheuma-

toid arthritis treated with either testosterone or dehydroepiandrosterone showed any objective clinical improvement. No clinical response was observed in the 2 patients (R.C. and R.MacD.) with cancer of the breast who received dehydroepiandrosterone. Of the 5 patients with cancer of the breast who received testosterone, 1 showed definite improvement (M.C.),

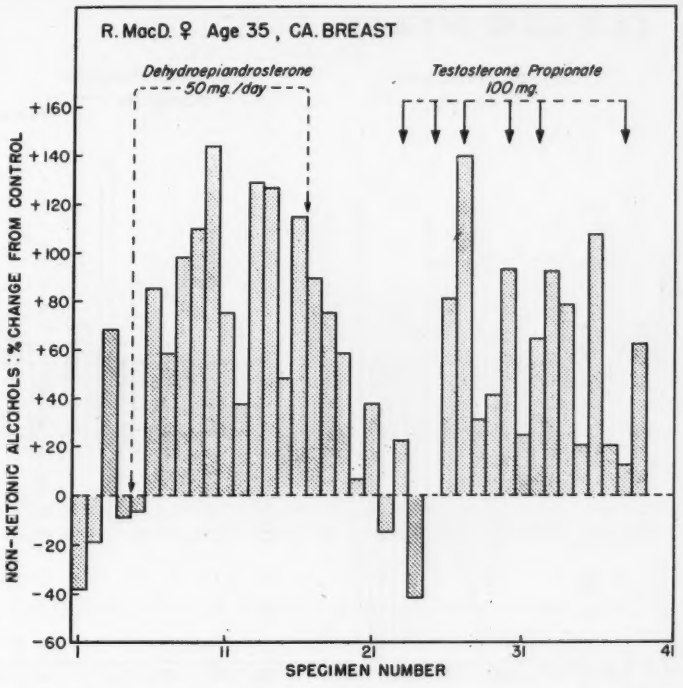

FIG. 5. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of nonketonic steroid alcohols in a 35-year-old woman with cancer of the breast who had been castrated (x-ray) two months previously.

1 had slight relief of pain (R.MacD.) and the condition of 2 deteriorated (R.C. and J.W.); there was clinical improvement in Patient F.R. later in the course of testosterone therapy at a time when no steroid studies were carried out. The patient with carcinoma of the prostate gland (J.S.) had increased pain and an elevation of serum acid phosphatase during the period of testosterone administration.

Within this small group of cancer patients, it is at present impossible to discern any correlation between steroid excretion and clinical response to androgen administration. The excretion of ketosteroids was elevated in all

of the patients who received androgen. Of the 2 patients whose clinical condition improved during the period of testosterone therapy, 1 (M.C.) showed a fall in nonketonic alcohol excretion and the other (R.MacD.) showed a rise in this component. In 2 of the remaining 3 patients, progression of the disease was observed. These 2 patients (R.C. and J.W.) showed

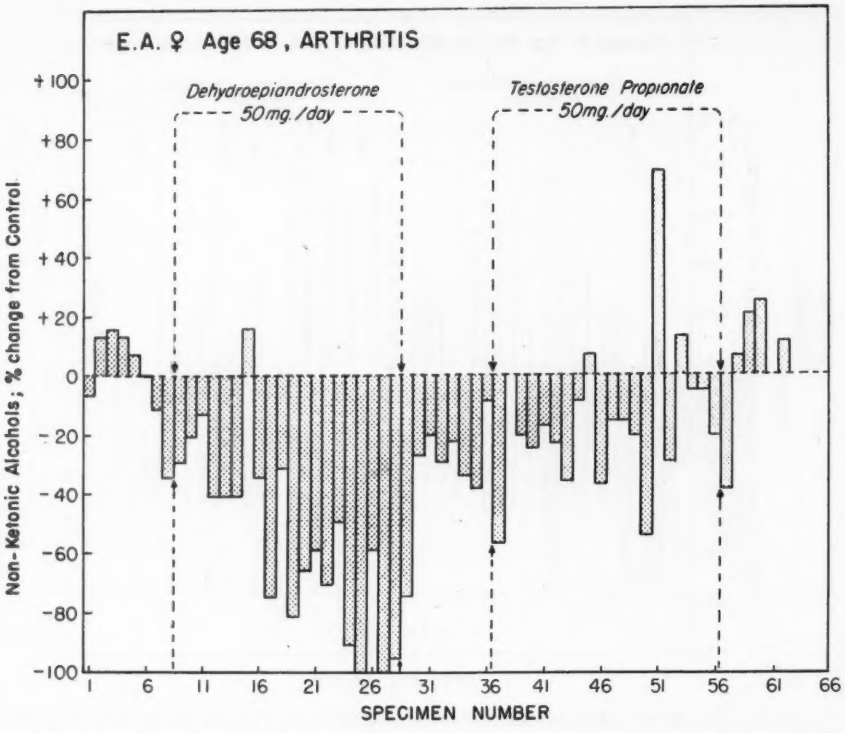

FIG. 6. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary excretion of nonketonic steroid alcohols in a 68-year-old woman with rheumatoid arthritis.

a decrease in the excretion of nonketonic alcohols but the other (F.R.) had an increase. Of the 2 patients who showed clinical improvement while under treatment with testosterone, 1 (R.MacD.) showed an increased excretion of estrogen, and the other (M.C.) no significant change. F.R., whose clinical condition improved later in the course of testosterone therapy, showed a markedly increased estrogen excretion. The other 3 whose clinical condition deteriorated during the experimental period also excreted significantly increased quantities of estrogen.

If the data on cancer patients are compared with those on the patients with arthritis (Table 1) several suggestive correlations emerge. Three of 4 patients with rheumatoid arthritis showed a fall in the excretion of nonketonic alcohols during testosterone therapy and the fourth showed no

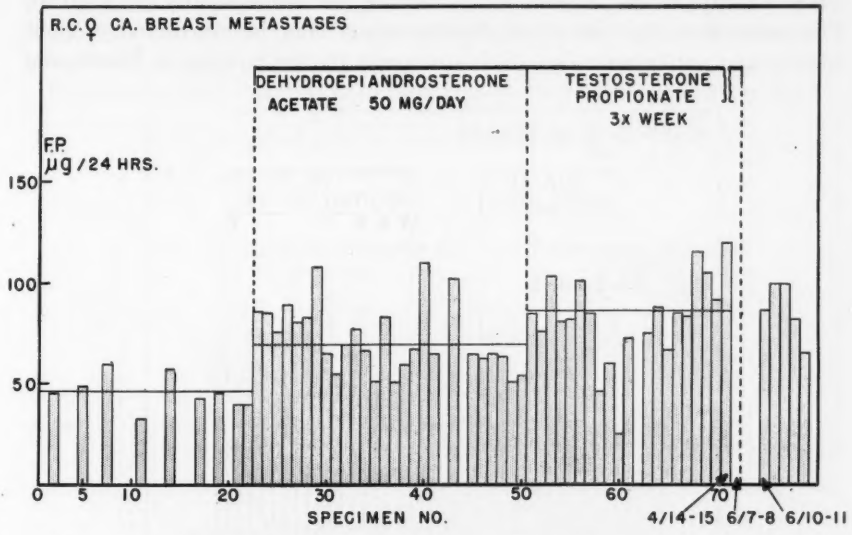

FIG. 7. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary FP excretion in a 69-year-old woman with metastatic carcinoma of the breast.

change. On the other hand, 3 of 6 cancer patients showed a fall and the remaining 3 a rise in nonketonic alcohol excretion. Only 2 of the 4 patients with rheumatoid arthritis showed an increased estrogen excretion following testosterone administration, whereas 5 of the 6 cancer patients showed such an increase. Alterations of steroid metabolism have been postulated both in cancer patients (7, 8, 9) and in patients with rheumatoid arthritis (8, 9). The results of the present investigation, though still scanty, tend to support this concept and also the ideas previously implied that the defects in steroid metabolism may be different in cancer and in arthritis.

Chemical findings: The increased excretion of ketosteroids after the administration of testosterone is in accord with the well established concepts of testosterone metabolism in the human (10). The major metabolites are androsterone and 5-isoandrosterone. These two ketosteroids are also the principal excretion products of administered dehydroepiandrosterone (11, 12). The variations in the excretion of nonketonic alcohols after the administration of dehydroepiandrosterone may be the resultant of two

phenomena: 1) the inhibition of the endogenous production of the precursors of the urinary nonketonic alcohols, and 2) varying metabolic pathways for the degradation of dehydroepiandrosterone. In the case of Patient R.C. both effects are discernible. These two types of reactions may occur to different extents when patients with varying metabolic status are studied. The possibility that dehydroepiandrosterone may be converted to both ketonic and nonketonic alcohols is supported by the findings of Mason and

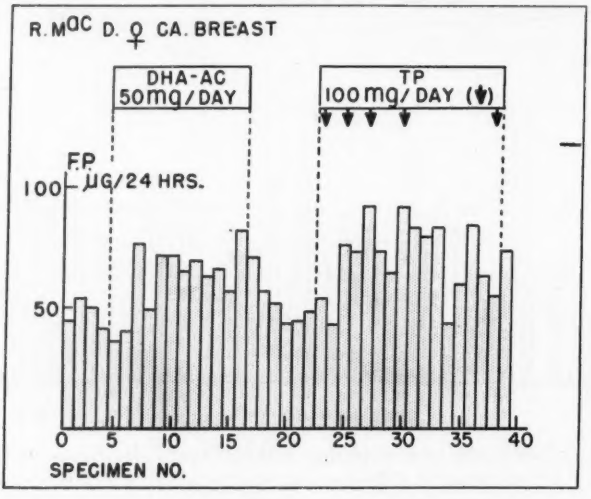

FIG. 8. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary FP excretion in a 35-year-old woman with cancer of the breast who had been castrated (x-ray) two months previously.

Kepler (13) that in a male with anterior pituitary insufficiency and in male and female patients with Addison's disease treated with dehydroepiandrosterone, the major urinary metabolites are androsterone, Δ^5-androstene-3β,17β-diol, and 5-isoandrosterone. However, Δ^5-androstene-3β,16α,17β-triol and several unidentified compounds accompanied them. In the urine of a male patient with Addison's disease who received dehydroepiandrosterone, Miller, Dorfman and Miller (12) found a small amount of androstane-3α,17β-diol in addition to androsterone and 5-isoandrosterone. The same authors reported that in addition to androsterone and 5-isoandrosterone a significant amount of Δ^5-androstene-3β,17β-diol was isolated from the urine of a normal woman who had been given dehydroepiandrosterone.

When testosterone is administered, it is reasonable to presume that the fall in nonketonic alcohol excretion is due to inhibition of the endogenous production of nonketonic alcohol precursors. The rises in nonketonic al-

cohol excretion which were observed in 3 of the 11 patients studied may be attributed to another metabolic pathway for this hormone. The striking effect, however, is the fall in nonketonic alcohol production when this compound is administered. This phenomenon may be akin to the depressant effect of methyltestosterone upon the urinary excretion of ketosteroids (14), which becomes apparent because methyltestosterone is not excreted as a ketosteroid.

Increased urinary excretion of biologically active estrogens after the ad-

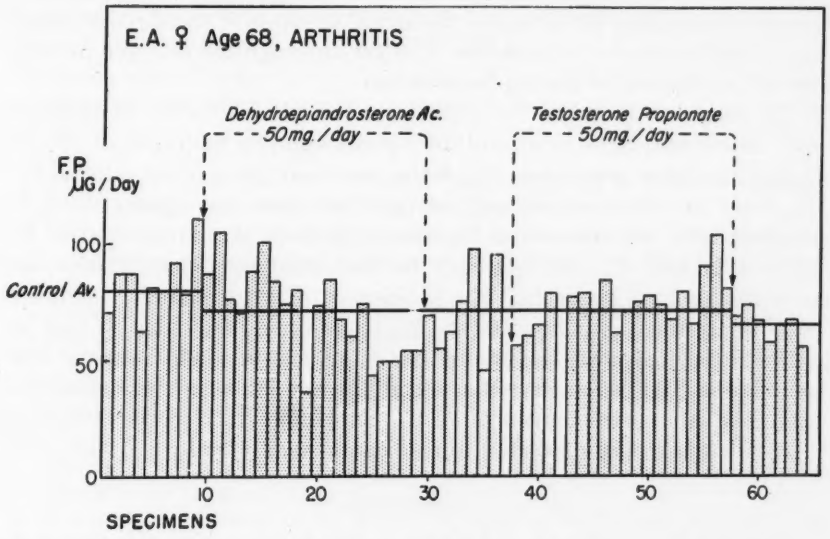

FIG. 9. The effect of the administration of dehydroepiandrosterone and testosterone on the urinary FP excretion in a 68-year-old woman with rheumatoid arthritis.

ministration of testosterone to normal males was observed as early as 1937 by Steinach and Kun (15). This finding has since been confirmed by others (16, 17, 18) who further showed that the estrogenic activity could be concentrated in the phenolic fraction. Nathanson and Towne (19) extended these observations to castrated women. In the present investigation, increased FP excretion was observed in the majority of the patients treated with both dehydroepiandrosterone and testosterone. In the 2 cancer patients who received both dehydroepiandrosterone and testosterone, it was possible to demonstrate by counter-current distribution that the increased FP excretion was due at least in part to an increased excretion of estrone and estriol, or estrone, estradiol and estriol (Table 2). It must be emphasized that the absolute FP level by itself is difficult to interpret. Although

appreciable amounts are excreted by untreated normal males and postmenopausal women, counter-current distribution studies have thus far failed to reveal the presence of estrone, estradiol-17β and estriol, although several components of the mixture have been characterized by partition coefficients. It is not known whether these materials are related to the known estrogens or are nonsteroidal in nature. However, despite this rather high base-line, it has been found that increases in FP titer are generally associated with an increased excretion of estrogen identifiable, at least in part, as one or more of the known urinary metabolites. The discrepancy between the increased FP titer and the actual amounts of the three estrogens found may be due to the presence of as yet unrecognized estrogen metabolites which respond to the fluorescence test.

The estrogens found in the urine after the administration of androgen may conceivably arise as a result of a stimulation by androgen or by synthesis from other precursors. The latter may take place in the adrenal cortex, since the increased urinary estrogen titer does not appear to be dependent upon the presence of functioning gonads. A more attractive hypothesis is that C_{19} steroids may be converted by demethylation and aromatization to estrogens. The occurrence of estrone and estradiol in large quantities in stallion testes (20) lends some credibility to this hypothesis. Furthermore, the aromatization of simple hydroaromatic compounds has been demonstrated in tissue slice and homogenate preparations (21). The laboratory conversion of C_{19} compounds to estrogens (C_{18}) (22, 23, 24) has been demonstrated to be a commercially useful process.

SUMMARY

1. Studies have been carried out on the urinary excretion of ketosteroids, nonketonic alcohols and FP after the administration of dehydroepiandrosterone to 2 cancer patients and 1 patient with rheumatoid arthritis, and after the administration of testosterone to 4 patients with rheumatoid arthritis and 7 cancer patients.

2. The excretion of ketosteroids was greatly increased in all cases after the administration of either hormone.

3. After the administration of dehydroepiandrosterone the excretion of nonketonic alcohols fell in the patient with rheumatoid arthritis and rose in the 2 cancer patients.

4. After the administration of testosterone the excretion of nonketonic alcohols fell in all 4 patients with rheumatoid arthritis.

5. The excretion of nonketonic alcohols fell in 4 and rose in 3 cancer patients treated with testosterone.

6. FP excretion rose in 2 of 3 male patients treated with testosterone.

7. FP excretion was unaltered in 1 female patient with rheumatoid

arthritis treated with dehydroepiandrosterone and testosterone.

8. FP excretion was increased in 2 female cancer patients treated with dehydroepiandrosterone and testosterone.

9. In the 2 female cancer patients treated with androgens the excreted estrogens were characterized, in one patient as estrone and estriol and in the other, as estrone, estradiol and estriol.

10. The data suggest that different errors in steroid metabolism may be involved in patients with breast cancer and in patients with rheumatoid arthritis. Their exact significance remains to be established.

REFERENCES

1. Nathanson, I. T.; Engel, L. L.; Kennedy, B. J., and Kelley, R. M.: Screening of steroids and allied compounds in neoplastic disease, *in* White, A.: Symposium on Steroids in Experimental and Clinical Practice. Philadelphia, The Blakiston Company, 1951, pp. 379–396.
2. Fieser, L. F., and Fieser, M., Natural Products Related to Phenanthrene, ed. 3. New York, Reinhold Publishing Corp., 1949, pp. v–viii.
3. Engel, L. L.; Patterson, H. R.; Wilson, H., and Schinkel, M.: The colorimetric estimation of urinary neutral steroid alcohols, *J. Biol. Chem.* **183:** 47–61 (March) 1950.
4. Nathanson, I. T., and Wilson, H.: Factors affecting colorimetric urinary 17-ketosteroid determinations, *Endocrinology* **33:** 189–203 (Oct.) 1943.
5. Wilson, H., and Nathanson, I. T.: The effect of alcohol and potassium hydroxide concentration on the reaction of 17-ketosteroids with m-dinitrobenzene, *Endocrinology* **37:** 208–216 (Sept.) 1945.
6. Engel, L. L.; Slaunwhite, W. R., Jr.; Carter, P., and Nathanson, I. T.: The separation of natural estrogens by counter-current distribution, *J. Biol. Chem.* **185:** 255–263 (July) 1950.
7. Dobriner, K.; Lieberman, S.; Wilson, H.; Ekman, B.; Pearson, O., and Eliel, L.: Adrenal function and steroid excretion in neoplastic disease, *in* Mote, J. R.: Proceedings of the First Clinical ACTH Conference. Philadelphia, The Blakiston Co., 1950, pp. 158–167.
8. Dobriner, K.; Lieberman, S.; Wilson, H.; Dunham, M.; Sommerville, I. F., and Rhoads, C. P.: Adrenal function and steroid excretion in disease, *in* Mote, J. R.: Proceedings of the Second Clinical ACTH Conference. Philadelphia, The Blakiston Co., 1951, Vol. 1, pp. 65–76.
9. Dobriner, K.: Adrenal function and steroid excretion in disease, *in* White, A.: Symposium on Steroids in Experimental and Clinical Practice. Philadelphia, The Blakiston Co., 1951, pp. 130–144.
10. Dobriner, K., and Lieberman, S.: The metabolism of steroid hormones in humans, *in* Gordon, E. S.: A Symposium on Steroid Hormones. The University of Wisconsin Press, 1950, pp. 46–86.
11. Mason, H. L., and Kepler, E. J.: Isolation of androsterone, etiocholan-3(α)-ol-17-one, and Δ^5-androstene-3(β),17(α)-diol from the urine after administration of dehydroisoandrosterone to a man, *J. Biol. Chem.* **160:** 255–264 (Sept.) 1945.
12. Miller, A. M.; Dorfman, R. I., and Miller, M.: Metabolism of the steroid hormones: metabolism of dehydroisoandrosterone, *Endocrinology* **46:** 105–110 (Jan.) 1950.

13. Mason, H. L., and Kepler, E. J.: Urinary steroids isolated after administration of dehydroisoandrosterone to human subjects, *J. Biol. Chem.* **167:** 73–76 (Jan.) 1947.
14. Reifenstein, E. C., Jr.; Forbes, A. P.; Albright, F.; Donaldson, E., and Carroll, E.: Effect of methyl testosterone on urinary 17-ketosteroids of adrenal origin, *J. Clin. Investigation* **24:** 416–434 (July) 1945.
15. Steinach, E., and Kun, H.: Transformation of male sex hormones into a substance with the action of a female hormone, *Lancet* **2:** 845 (Oct. 9) 1937.
16. Callow, N. H.; Callow, R. K., and Emmens, C. W.: The effect of the administration of testosterone propionate on the urinary excretion of compounds allied to the steroid hormones, *J. Endocrinol.* **1:** 99–107 (June) 1939.
17. Dorfman, R. I., and Hamilton, J. B.: The urinary excretion of estrogenic substances after the administration of testosterone propionate, *Endocrinology* **25:** 33–38 (July) 1939.
18. Hoskins, W. H.; Coffman, J. R.; Koch, F. C., and Kenyon, A. T.: The effect of testosterone propionate on the urinary excretion of androgens and estrogens in eunuchoidism, *Endocrinology* **24:** 702–710 (May) 1939.
19. Nathanson, I. T., and Towne, L. E.: The urinary excretion of estrogens, androgens and FSH following the administration of testosterone to human female castrates, *Endocrinology* **25:** 754–758 (Nov.) 1939.
20. Beall, D.: The isolation of α-oestradiol and oestrone from horse testes, *Biochem. J.* **34:** 1293–1298 (Sept.) 1940.
21. Beer, C. T.; Dickens, F., and Pearson, J.: The aromatization of hydrogenated derivatives of benzoic acid in animal tissues, *Biochem. J.* **48:** 222–237 (Feb.) 1951.
22. Inhoffen, H. H., and Zühlsdorff, G.: Übergang von Sterinen in aromatische Verbindungen. VI. Mitteil. Die Darstellung des Follikelhormons Oestradiol aus Cholesterin, *Ber. d. deutsch. chem. Gesellsch.* **74:** 1911 (Dec.) 1941.
23. Wilds, A. L., and Djerassi, C.: The synthesis of estradiol and 1-methyl-estradiol from cholesterol, *J. Am. Chem. Soc.* **68:** 2125–2133 (Nov.) 1946.
24. Hershberg, E. B.; Rubin, M., and Schwenk, E.: Synthesis of estrone from androstadienedione, *J. Org. Chem.* **15:** 292–300 (March) 1950.

SOME BASIC HORMONE PROBLEMS*

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SINCE this is the first official occasion at which the Endocrine Society meets under its new name, it is perhaps appropriate to cast a look backward at fundamental scientific problems with which it has been concerned and to look forward to problems which it will face. It is the great good fortune of science that no ultimate word is ever said, and when one considers the present state of the science of endocrinology it is obvious that we are not even approaching the antepenultimate word. For we are in the midst of a period of expansion and inquiry into all aspects of endocrinology. To attempt to appraise all of the basic problems of the multifaceted activity requires a temerity which I do not possess; so I shall in fact attempt to discuss *some* basic problems.

What are these basic hormone problems? There are four which persistently intrude, no matter what aspect of hormone research one examines. The first of these is concerned with the genesis and fate of internal secretions. The second involves a consideration of the mechanism of the action of the hormones in both health and disease, particularly at the cellular level where all vital processes originate. The third is the often mentioned, much discussed problem of hormonal balance and the means whereby it is effected. The fourth is the superficially narrow but potentially enormous question of the relationship between the two great regulating systems of the body, the endocrine and the central nervous systems. Past investigations have furnished a more or less solid substratum for future attack upon these problems, but I am sure we shall all agree that major questions are still unanswered. In presenting to you a few thoughts about them, I hope you will forgive me if I restrict myself to those phases with which I have some degree of familiarity. I shall neglect, therefore, chiefly out of ignorance, organisms and hormones which are just as significant as those discussed.

THE GENESIS AND FATE OF INTERNAL SECRETION

In considering the origin of hormonal secretions one is faced with two challenging sets of facts. First of all the chemical nature of hormonal sub-

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stances is remarkably variable, depending upon the site of their synthesis. Hormones are unpredictably constituted; they may be proteins, steroids, aromatic amines or polypeptides. Secondly, the chemical nature of the majority of hormonally active substances is now rather well known. The structural formulae of many are now textbook commonplaces, and even the more complex protein hormones may be characterized by numerous physicochemical and other analytic criteria. The wealth of organic chemical data available to us is matched by a poverty of knowledge concerning the chemical precursors of the active hormones, particularly of those hormones which are so powerfully active in the bodies of the higher animals. Our knowledge of the chemical nature of these substances has been advanced by a series of brilliant chemical attacks upon the tissues from which the hormones originate, and the purification and/or isolation of the active substances. Final proof of chemical structure has been afforded by either partial or total synthesis of hormonal isolates. For example, there is no more remarkable feat in the realm of organic chemistry than the identification and synthesis of the major active stercid hormones by that group of great chemists including Doisy, Bachmann, Kendall, Reichstein, Laqueur, Ruzicka, Julian, Gallagher, Sarett, Woodward and many others. It seems to me that the focusing of similar talents upon the problem of hormone biogenesis must result in an era as fruitful as that which these men have just created.

Particularly propitious to the study of hormone biosynthesis is one great facility recently made available, namely, a relative plenitude of isotopes of the atomic constituents of hormone molecules, along with refined means for isotope identification. Coupled with the availability of isotopic techniques is the recent development of excellent microanalytic methods in both organic and physical chemistry, such as paper chromatography, partition chromatography, infrared spectrophotometry, and even microwave analysis. Finally, the resources of the physiologist and biochemist in devising means for the study of the secretory organs and tissues themselves are constantly being developed.

You will pardon me if I cite as an example of the means of attack, some work from our laboratories in Shrewsbury. We have been concerned with the biogenesis of steroid hormones from the adrenals and gonads. Taking advantage of the finding by Hechter and his collaborators (1) that the isolated, perfused gland will produce steroid hormones, we have demonstrated that adrenocorticotropic hormone perfused through the adrenals will significantly promote the production of a variety of corticosteroids by bovine, pig, dog and human adrenals. Gonadotropin will similarly stimulate androgenic steroid production by the isolated perfused testis. By perfusing appropriate steroid precursors through the adrenal glands, typical

transformation reactions have been demonstrated, such as specific oxidations at carbons 3, 11, 17 and 20, and the conversion of α,β unsaturated ring A ketone to the 3β-hydroxy derivative with the characteristic allo configuration. The hormonally active end products of corticosteroidogenesis are typified by 17-hydroxycorticosterone and corticosterone; by perfusing C14-labeled cholesterol we have obtained these as radioactive compounds, demonstrating cholesterol as a parent compound. Furthermore, following perfusion with radioacetate, these same corticosteroids emerge with radioactivity pointing to their origin from a two-carbon precursor. In like manner demonstration of the synthesis of radioactive testosterone and Δ^4-androstenedione has been made by testis perfusion with radioacetate; and we have preliminary evidence of similar ovarian hormone synthesis. Following, and along with, these demonstrations of steroidogenesis by isolated perfused glands have come studies in a number of laboratories of enzymatic systems responsible for the observed transformation. ACTH and gonadotropin act upon adrenal and testis slices respectively to stimulate production of the typical hormonal end products. Characterization of the enzyme systems responsible for specific oxidations is well under way.

What I have thus summarily described for steroidogenesis raises more problems than it answers. The intermediate steps leading from acetate to corticosteroid, androgen, or estrogen are completely unknown. We know that in part acetate is synthesized to cholesterol, which in turn is degraded to hormonal steroid; but a more direct synthetic pathway is also suggested by our data. A fascinating series of linked enzymatic transformations is suggested. If this fragmentary knowledge of steroid hormone anabolism represents our present state in this field, we are even more unenlightened when it comes to other hormones. I suggest that the tools for discovery are available, and that the doing awaits their use.

Our knowledge of the chemical fate of secreted hormones within the body is also fragmentary. One of the most fundamental of all observations is that any hormone injected into the blood stream disappears with extraordinary rapidity. This disappearance may involve its chemical transformation in the blood itself. For example, yesterday we described an enzymatic system in blood that inactivates ACTH (2). There is evidence for similar inactivating systems for gonadotropin, epinephrine, or estrogen. In addition certain hormones are acted upon by detoxifying mechanisms, with consequent excretion of the detoxified form—outstanding examples are the urinary steroid glucuronides. Finally, fairly specific metabolic transformations occur in numerous tissues of the body.

Whatever the metabolic fate of circulating hormone may be, the basic mechanism involved is an enzymatic one. The hormones act as substrates. The chemical history of these substrates is partially known in certain in-

stances. The pathways leading to the transformation of testosterone to certain major steroidal end products have, for example, been fairly well established. Similar data, more or less complete, are available for other steroid hormones; but for no single steroid hormone has a complete balance sheet been made. Thus, in one of the best known instances, pregnanediol accounts for, depending on the type of subject investigated, from 10 per cent to 40 per cent of the progesterone administered. The missing 60 per cent to 90 per cent is the unknown. Recent data of Heard (3) and his collaborators suggest a great variety of transformation products, since the administration of C^{14}-progesterone led to recovery of radioactivity in a number of lipid as well as aqueous fractions from urine and feces.

Studies with isotope-labeled hormones suggest not only a diversity of catabolic products but also certain interesting sites of hormone metabolism, such as the relative concentration of estrogen in mammary glands, or of growth hormone in the pancreas. Does this suggest special transformation occurring in special target organs? We have recently, for example, identified six transformation products of 17-hydroxycorticosterone following its injection into arthritic joints, whereas following its perfusion through the liver a much larger number of metabolites are obtained. Differences between the actions of various tissues on hormonal substrates may be multiplied. Do these differences imply utilization of special metabolites for specific end-organ effects?

THE MECHANISMS OF HORMONE ACTION

The question which I have just asked involves us at once with problems of the mechanism of hormone action. If we consider the great assembly of hormonal substances we are struck by the fact that, with very limited exceptions, a known secretory product exerts diverse characteristic effects. Thus epinephrine administration leads not only to vasoconstriction in some tissues, but it causes vasodilation in others; it speeds the heartbeat, causes sweating, pilomotor erection, and an eosinopenia. Estradiol causes uterine vasodilation, water imbibition and growth; it induces epithelial growth and desquamation in the vagina, it affects osteogenesis, mammary development, achilles tendon reflex time, lymphatic tissue, the nasal mucosa, duodenal contraction, hair growth and even dreams. One possible basis for this diversity of effect may involve the action of a specific transformation product in one site and of a different transformation product in another site. The fact that cortisone is inactive upon direct administration into the human skin or into an arthritic joint, whereas it is active upon both of these sites following systemic administration, suggests that transformation products must be the locally effective substances in these tissues; and in-

deed there is good evidence that 17-hydroxycorticosterone is at least a major effective transformation product in this instance.

Considering what is the locally active substance in any given hormonal effect merely initiates the question of how it acts. Here again the pat answer is that it acts by way of an enzyme system. Yet when we consider the numerous attempts to demonstrate the role of hormonally active substance in a host of known enzyme systems we are confronted with instance after instance of bafflement. The glycogenetic action of insulin has been attributed to the inhibition by insulin of an inhibitor of the enzyme hexokinase. But attempts to identify the hexokinase inhibitor have led to peculiar conclusions: It may be a pituitary hormone but it is no known pituitary principle; it may be found in adrenal extract but is no known corticosteroid. Hayano and Dorfman (4) have shown that the inhibitory effects of various active neutral steroids upon the apoenzyme of d-amino oxidase are real and demonstrable with the isolated enzyme system. Nonetheless, the correlation of this effect with specific activities of these hormones has not been accomplished. Hochster and Quastel (5) have demonstrated similar effects *in vitro* of ketonic steroids upon a glycerophosphatase system, but again specificity is lacking in terms of the hormonal actions of the active substances.

If the data on effects *in vitro* upon certain enzyme systems leave much perplexity, we are even more at sea when we consider effects upon known enzyme systems *in vivo*. For here, time and again we observe effects which are sometimes quite specific *in vivo*, but which cannot be duplicated *in vitro*. Thus cortisone rather specifically affects proline oxidase activity, but no direct effect upon the isolated enzyme system has been demonstrated (6). The concentrations of liver and kidney arginase are affected by pituitary growth hormone and by various steroids, but no action on arginase *in vitro* has been shown (7). The suggestion has indeed been made by Kochakian that it is the synthesis of enzyme by tissue which is hormone-conditioned, rather than any involvement in enzymatic activity as co-factor. This does not seem likely as an over-all explanation of hormone-enzyme relationships, since the almost instantaneous effects of certain hormones would seem to rule out stimulation of enzyme synthesis to the extent called for by the quantitative results achieved.

By what mechanisms then do hormones exert their characteristic effects on cells and on cellular processes? There have been persistent suggestions of activity at cell surfaces—activity which may affect cellular permeability as well as the linkage to the cell surface of secondary or tertiary substances. The demonstration that progesterone permits the passage through uterine endometrium of substance which is growth-promoting to the blastocyst, is

perhaps another example (8); and the adsorption of insulin to the diaphragm suggests a specific linkage to cell membrane (9). These represent, however, merely invitations to exploration. When one considers the energetics involved in hormone-conditioned processes, with rates of reaction involved varying from millisecond effects (*e.g.*, at synapses) to growth processes lasting many days, it would seem that no general explanation can be had. Rather, the painstaking unravelment of specific reaction effects appears called for in our present state of ignorance.

HORMONAL BALANCE

The hormones acting at the cellular level do not initiate movement down one-way streets. Specific hormones condition reactions which, in turn, may be conditioned by other specific hormones. This is an observation almost as old as endocrinology itself. The hypothyroid individual does not respond adequately to a variety of internal secretions; excessive adrenocortical secretion prevents the action of pituitary growth hormone. At this meeting we hear how known estrogenic substances synergize with each other (10). The instances of hormone antagonism, interaction and synergism are multiplied week by week with each publication of articles on endocrinology. Conditioning of hormone by hormone at the cellular level is paralleled by conditioning of hormone secretion. The inhibition and/or stimulation of pituitary tropic hormone secretion by a variety of steroids are familiar phenomena. Neurohumoral conditioned release of a variety of internal secretions is well known. The secretory equilibria which subsist from moment to moment, day to day, and year to year, represent a marvelous and intricate mechanism of checks and balances.

In spite of numerous detailed descriptions of various phases of these balance systems there are certain basic problems evident to the thoughtful examiner. Let us look first at the secretory equilibrium. We ordinarily consider that its moment-to-moment maintenance involves a sort of servomechanism. Pituitary gonadotropin, for example, causes the secretion of gonad steroid which, in turn, will suppress excess gonadotropin secretion by the pituitary. Somewhat closer examination of experimental facts suggests that gonadotropin release in the female may be elicited by estrogen circulating at certain levels, with suppression occurring at higher levels or with more prolonged circulation. Even closer analysis suggests that gonadotropin release involves FSH secretion being affected by one set of steroidal products and LH release by other substances or combinations thereof. Finally, as Markee and his collaborators (11) have so painstakingly shown, an effect of steroid upon hypothalamus must be interpolated between the gonads and the pituitary.

What are the problems inherent to such a self-regulating system? There are two which continually face us: 1) The problem of utilization of the secretion regulators, and 2) the problem of maintenance of the secretory systems involved. Here at this meeting Mortimore and Heller have presented us with an instance of utilization of gonadotropin by the testes (12). Some gonadal mechanism presumably fixes the gonadotropin within the tissue and uses it up. How is this fixation effected? We do not know. To maintain responsiveness to the inhibitors and stimulators, each secretory mechanism must be maintained at a certain level of activity. The attainment of a given level of activity requires energy. Where does the energy come from? Do steroids acting upon the hypothalamus furnish the energy for its humoral release system? We may say glibly that secretory cells are maintained by the ordinary energy-producing, oxidative, or glycolytic systems which underlie cellular activities generally. But is this so? Does secretion require the supplying of special nutritive substances now unknown, and demanding characterization?

These considerations of the utilization of secretion inducers and the maintenance of secretory systems apply also to hormonal balancing at nonsecretory end organs. Does the antagonism of certain hormones involve competition for cellular acceptors? Does synergism require the supplying of specific substances to certain limiting reactions by one synergist and of other specific substances by the other?

One final aspect of the problem of hormone balance involves the year-to-year equilibria. What, in short, happens to the hormone balance in the growing adult and aging organisms? We have had a number of instances of the quantitative increase and decline of steroid secretions in youth and age. Urinary ketosteroids, for example, rise to a maximum in young men and women, and decline quantitatively thereafter (13). The rise and fall of estrogen excretion with age in women is well documented. But estrogen excretion in men appears to be constant from decade to decade (14). What are the consequences of these alterations in balance? To what extent are the processes of aging conditioned by these alterations? Gropingly, geriatric practice attempts the elevation of circulating androgen in aging men, or of estrogen in aging women. Surely this is a naive approach. We have progressed from opotherapy to the administration of powerfully active compounds; but is this progress, if not compounds but complex balances are involved?

ENDOCRINE-CENTRAL NERVOUS SYSTEM RELATIONSHIP

Hormone balances within the organism condition the activity of every tissue of the body. Today there is scarcely a tissue which has not been dem-

onstrated as a target organ for several hormones. We may proceed from the integument to the gut, from the heart to the skeleton, and in each we find hormone-conditioned processes. The central nervous system, superficially, is most exempt from endocrine influences. The blood-brain barrier presumably prevents access of circulating internal secretions along with other substances having biologic activities in more accessible tissues. A number of ancient observations are regularly cited as mitigants of this apparent inviolability—the mentality of the cretin, the effects of estrogens on specific reflex phenomena, the hyperirritability of the hyperthyroid subject, and the specific humoral mechanisms involved in sympathetic parasympathetic conduction.

The nervous factors involved in hormonal secretion have, of course, been extensively investigated in the classic works of Cannon on adrenomedullary secretion and more recently in studies of the hypothalamic control of pituitary secretion. That many secretory processes are exempt from nervous control has been shown by anatomic and neurophysiologic investigation over many years. Here, as in every problem, we have discussed, we observe no general rule underlying neurohumoral relationships, but rather specific instances of nervous activities regulating hormonal secretion and of hormonal secretions affecting specific nervous organs.

Nonetheless, some recent observations give point to a more intensive examination of neuroendocrine relationships. I refer to the evidence that certain life stresses having their immediate impact upon the central nervous system induce somatic repercussions which are endocrine-conditioned. A simple example is hysterical amenorrhea in which anxiety, conscious or unconscious, inhibits, by way of a hypothalamic mechanism, pituitary gonadotropin secretion (15). More complex and still problematic are a large group of psychosomatic symptoms based on neurotic and psychotic derangements. I cite the evidences of hypoadrenalism in certain schizophrenias and of hyperadrenalism in certain manic states. The induction of psychotic behavior in certain cortisone or ACTH-treated patients indicates the possibility of back reactions upon the central nervous system. That such back reactions may have a substantial physiologic basis is suggested by the work of Hoagland and his collaborators who have demonstrated significant delay in synaptic conduction on the one hand, and reduced cerebral circulation on the other hand, in adrenalectomized animals (16). Replacement therapy with various steroids effectively restores these to normal function.

Are we perhaps moving into an era in which a more detailed study of nervous functioning may reveal hitherto unsuspected conditioning by those molecular species called hormones? Is mental health based at least in part on hormonal health? I think these questions may be answered only by the critical investigation of the basic phenomena involved.

BASIC HORMONE PROBLEMS

I have perhaps raised too many problems and asked too many questions. Moreover, in attempting in one-half hour even a cursory analysis of some basic hormone problems, a most incomplete appraisal is unavoidable. What to some may appear essential, is neglected. Nonetheless, if I have stimulated cerebration leading to action, it is enough.

Year after year I have come to these meetings of our Society and, after patient listening, have emerged somewhat dazed by the wealth of data and ideas presented. At the end of each meeting I have thought that now this problem or that problem has been solved. No more need be said. But when I return the next year I am greeted by even more illumination, by fresh, surprising and ingenious discovery. How remarkable, how endless is our opportunity! And because of this year's advances, of past years' advances and the advances which are inevitable in the years before us, men and women all over the world must of necessity be benefited. It is, therefore, appropriate to discuss basic hormone problems, for they are germane to human health and to human happiness.

REFERENCES

1. Hechter, O.; Zaffaroni, A.; Jacobsen, R. P.; Levy, H.; Jeanloz, R. W.; Schenker, V., and Pincus, G.: The nature and biogenesis of the adrenal secretory product, *Recent Progr. Hormone Res.* **6**: 215, 1951.
2. Pincus, G.; Hechter, O., and Hopkins, T.: The inactivation of ACTH in mammalian blood, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 920, 1952.
3. Heard, R. D. H.; Peron, F.; Solomon, S., and Thompson, L.: in *Proc. Canad. Physiol. Soc.* October 1951.
4. Hayano, M., and Dorfman, R. I.: Studies on the inhibition of various enyzmes by steroids, *Ann. New York Acad. Sc.* **54**: 608, 1951.
5. Hochster, R. M., and Quastel, J. H.: Effects of steroids and diethylstilbestrol on dehydrogenase systems, *Ann. New York Acad. Sc.* **54**: 626, 1951.
6. Umbreit, W. W.: The influence of adrenalectomy and cortisone treatment on enzymatic reactions in rat tissues, *Ann. New York Acad. Sci.* **54**: 569, 1951.
7. Kochakian, C. D.: Protein anabolic effects of steroid hormones, *Vitamins and Hormones* **4**: 255, 1946.
8. Pincus, G.: Factors controlling the growth of rabbit blastocysts, *Am. J. Physiol.* **133**: 412, 1941.
9. Stadie, C. W.: The combination of insulin with tissue, *Ann. New York Acad. Sc.* **54**: 671, 1951.
10. Grauer, R. C.; Cutuly, E., and Saier, E.: The influence of mixtures of biologically active estrogens on target organ response, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 947, 1952.
11. Markee, J. E.; Everett, J. W., and Sawyer, C. H.: The relationship of the nervous system to the release of gonadotrophin and the regulation of the sex cycle, *Recent Progr. Hormone Res.* **7**: 139, 1952.
12. Mortimore, G. E., and Heller, C. G.: Germinal cell arrest and germinal cell "hyperactivity" in relation to the theory of utilization of gonadotropins, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 952, 1952.

13. Dorfman, R. I.: Biochemistry of androgens, *The Hormones* **1**: 467, 1948.
14. Pincus, G.; Romanoff, L. P., and Carlo, J.: Urinary steroid excretion in relation to age, *J. Gerontol.* **6**: 135, 1951.
15. Klinefelter, H. F., Jr.; Albright, F., and Griswold, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3**: 529, 1943.
16. Bergen, J. R.; Hunt, C., and Hoagland, H.: Blood flow and oxygen consumption of brain in relation to adrenal cortical action, *Fed. Proc.* **11**: 12, 1952.
Slocombe, A.; Hoagland, H., and Praglin, J.: Conduction time in the central nervous system and peripheral nerve excitability in relation to adrenal cortical function, *Ibid.* p. 149.

BLOOD RADIOIODINE CONCENTRATION AND BLOOD RADIATION DOSAGE DURING I^{131} THERAPY FOR METASTATIC THYROID CARCINOMA*

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TO DETERMINE the radiation dosage delivered to the blood as well as to the whole body during radioiodine therapy, studies were made of the blood I^{131} concentration following 53 oral therapeutic doses to 23 patients with metastatic thyroid carcinoma. Data were obtained for four days or more after ingestion of the isotope.

As a result of the administration of I^{131}, radiation is delivered not only to the thyroid tissue, which concentrates radioiodine, but also, in smaller amounts, to the blood and to the whole body. Radioiodine therapy is clinically expedient only when the radiation dosage to the thyroid gland or to the thyroid tumor greatly exceeds that to the blood. The radiation dosage rate to the blood may be calculated, as shown subsequently, from its I^{131} concentration and from the disintegration scheme and physical half-life of the isotope. The ratio between the generalized body radiation and that delivered to the blood will approximately equal the ratio of the average concentration of radioiodine in the body to its concentration in whole blood.

TECHNIQUE AND CALCULATIONS

The radioiodine concentration in blood was determined by gamma-ray counting of the radioactivity of weighed oxalated samples of whole blood. Several specimens were taken during the first day after ingestion, at which time the I^{131} level is highest and is changing most rapidly. Subsequent samples were drawn with decreasing frequency for periods which ranged from four to thirty-five days.

Using the disintegration scheme for I^{131} proposed by Metzger and Deutsch (1) and taking 93 ergs/Gm. as equal to 1 roentgen equivalent physical (REP)(2), it is found that a radiation rate of .451 REP/hr. results from the beta rays within blood or tissue containing a uniform concentration of 1 microcurie of I^{131} per gram.

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We have computed the radiation dosage delivered to the blood by the gamma rays from I^{131}. These calculations were based on the values which Bush (3) obtained for the dosage rate delivered to the body when it contains a uniform concentration of an isotope emitting gamma rays. Following the methods of Marinelli, Quimby and Hine (4), we have determined that the gamma rays from I^{131} deliver 2.41 r/mc. hr. at 1 cm. When this value is used in Bush's calculations, the results show that the body of an average-sized patient (70 Kg. weight, 170 cm. height) would receive radiation at an average rate of .294 r/hr. if it contained a uniform concentration of 1 microcurie of I^{131} per gram of tissue.

The dosage rate, due to both gamma and beta radiation, is the sum of the two dosage

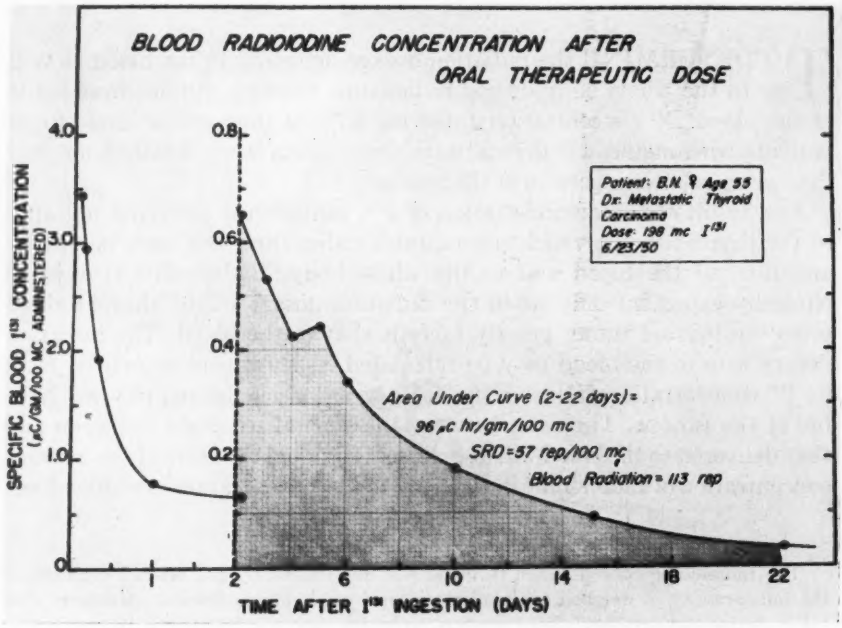

FIG. 1. Representative plot of the specific I^{131} concentration in whole blood.

rates and equals .451 C_b+.294 C_t, where C_b is the concentration of I^{131} in whole blood (μc/Gm.) and C_t is the average concentration in body tissue.

Since C_b is directly measured, it is desirable to determine, if possible, the ratio between C_b and C_t. Actually, this ratio between the blood concentration of I^{131} and its concentration in tissue is a complex function of time, which depends markedly on the fraction of the circulating I^{131} which is organically bound. It is possible, however, to make a rough estimate of the blood/tissue ratio for the period shortly after ingestion, when only radioiodide will be found. Scott *et al.* (5) found that during this period the I^{131} concentration in erythrocytes is approximately half that in plasma. For a patient with a hematocrit of 50 per cent, this value would give a blood concentration of about $\frac{3}{4}$ of the plasma

concentration. Myant *et al.* (6) obtained an erythrocyte/plasma radioiodide ratio of about $\frac{2}{3}$. They and Berson *et al.* (7) have shown that the iodide space in equilibrium with blood plasma after administration of I^{131} is about $\frac{1}{3}$ of the body mass—*i.e.*, that the average tissue concentration of radioiodide is about $\frac{1}{3}$ of the plasma concentration. From this, it is seen that the blood concentration may be about two or three times the tissue concentration, during the period when the I^{131} is found as an inorganic iodide. Following release of organically bound I^{131}, one may expect (8) that its concentration in blood will become even higher relative to tissue.

However, in this study, to facilitate calculation and to provide a reasonable upper limit to the radiation dosage delivered, it will be assumed, on the basis of the preceding considerations, that the ratio of the average I^{131} concentration in body tissues to that in blood has a constant value which will be taken as $\frac{1}{2}$, *i.e.*, $C_t = \frac{C_b}{2}$

Using this assumption, one obtains the following relationship:

$$\text{(A) Dosage rate to blood } \frac{(\text{rep})}{\text{hr.}} = .451\ C_b + .294\ C_t$$

$$= .451\ C_b + .294 \frac{C_b}{2} = .598\ C_b$$

The specific radiation dose (SRD) to the blood, *i.e.*, the radiation delivered in a given time interval per 100 mc. of I^{131} administered, can be calculated from a plot of the specific I^{131} concentration (μc./Gm./100 mc. administered) against time. A representative plot is shown in Figure 1. By using the following relation, the SRD for any desired time interval is determined from the area under the curve.

(B) Specific radiation dosage = Radiation delivered per 100 mc. of I^{131} administered

$$= .598 \frac{\text{Gm. rep}}{\mu\text{c. hr.}} \times \text{area under specific blood } I^{131} \text{ concentration curve } \frac{(\mu\text{c./Gm.})(\text{hr.})}{(100\ \text{mc.})}$$

The radiation delivered during the period after the time of withdrawal of the last blood sample is calculated using an eight-day physical half-life for I^{131} and the assumption that the isotope within the blood is in biologic equilibrium, *i.e.*, that changes in its concentration are due only to physical decay. This convenient assumption, although not valid in general, will usually result in only a small error in the total SRD. The error is particularly small when the I^{131} concentration in the terminal sample is relatively low. Under these conditions the SRD delivered from the time of the last blood sample to the time when the blood no longer contains I^{131} will equal 165 times the last observed specific I^{131} concentration.

The radiation delivered following administration of a given dose of I^{131} is the product of the specific radiation dose and the number of 100 mc. of I^{131} administered. Figure 1 illustrates the calculation for a representative dose to a patient. Using the assumption employed in this study, that $C_t = \frac{1}{2}C_b$, the generalized body radiation delivered during any period will equal one half the radiation delivered to the blood. An identical ratio will, of course, be found for the specific radiation dosage to the body.

RESULTS

The specific radiation dosage delivered from the time of ingestion of the isotope until the radioiodine concentration in the blood is negligible, has varied from 22 to 182 REP per 100 mc. administered. The average value was 52 ± 31** REP/100 mc. Marinelli and Hill (9) in a similar study of 32 doses to 20 patients report an average SRD of 108 REP/100 mc. Their average SRD, when modified to conform to the physical assumptions and factors used in the present study, equals 66 REP/100 mc.

The radiation following a single therapeutic dose has been calculated to be as high as 320 REP to the blood, and half as high to the whole body, if we assume—as we do—that the blood I^{131} concentration is twice that in all tissues. This radiation was delivered after the administration of 208 mc. of I^{131}, the largest single dose given to a patient (B.N.) in our series. Following this dose, the second largest generalized body SRD in our series was delivered (77 REP/100 mc.).

The generally accepted value for the median lethal dose of external radiation to man is 400r, when delivered within a very short time (10). To deliver an equivalent amount of radiation to the body of even this patient (B.N.) would probably require about 600 mc. of I^{131}—a quantity of I^{131} several times higher than our usual dosage (100–120 mc.). It is also probable that the LD_{50} for radiation from I^{131} would be higher than 400 REP, since with this isotope the radiation is delivered over a period of approximately two weeks.

It is interesting to note that clinically, consistent with the above considerations, no significant untoward effects followed a single therapeutic dose in our series of patients, except for temporary leukopenia. On the other hand, in 2 out of 13 patients extensively treated with radioiodine for metastatic thyroid carcinoma persistent leukopenia developed. One of these patients received 1,000 mc. over an eight-month period, resulting in the delivery of about 1,200 REP. Leukopenia developed in the other patient after administration of 450 mc. of I^{131}, which delivered a total of *ca.* 200 REP over a six-month interval. The latter estimate was based on only one blood curve. One of our patients died in terminal leukemia.

EFFECT OF THYROIDECTOMY ON SRD

The effect of thyroidectomy on radiation delivered to the blood of 5 patients was studied. For these patients, blood curves were available on the I^{131} dose employed to effect a radiation thyroidectomy and for a total of 11 post-thyroidectomy therapeutic doses. Blood concentrations were deter-

** Standard deviation.

mined for intervals varying from four to twenty-three days following I^{131} administration.

The SRD for each dose was determined for three periods: (a) the period of radioiodine absorption by the thyroid, which we have taken as the initial forty-eight hours after I^{131} ingestion; (b) the period of radioiodine release by the thyroid, *i.e.*, the interval following the initial two days; and (c) the total time of irradiation. The SRD delivered during (c), the entire period, is the sum of that delivered during the period of radioiodine absorption and the period of radioiodine release. For every patient and for each of these three intervals, a comparison was made of the SRD delivered by the thyroidectomizing dose and by subsequent therapeutic doses of I^{131}.

Changes during the first period, the absorption phase, primarily reflect changes in the pattern of iodide removal from the blood by the thyroid gland. A rise or fall during the second period is related to a change in the pattern of release of I^{131} to the blood stream from the thyroid. The third value indicates changes in the over-all pattern.

The results of this study are given in Table 1. They show that the blood radioiodine concentration, and hence the associated SRD, is nearly always

TABLE 1. EFFECT OF THYROIDECTOMY ON SPECIFIC RADIATION DOSAGE

Period	Doses		Patients	
	Increased SRD	Decreased SRD	Increased SRD	Decreased SRD
I^{131} absorption (0–2d)	10	1	4	1
I^{131} release (after 2d)	3	8	1	4
Total irradiation time	3	8	1	4

lower during the absorption period of a thyroidectomizing dose than during the same interval after a therapeutic dose to the same patient following ablation of the thyroid. This phenomenon can be explained by consideration of the metabolism of the administered radioiodide. Radioiodide clearance from the blood is performed primarily by the thyroid gland or functioning thyroid metastases and by the kidneys. When the thyroid gland is removed, the administered iodide will remain in the blood for a longer period, thus increasing the I^{131} concentration and, secondarily, the radiation delivered to the blood. This increase in blood radioiodine concentration may be partially responsible for the induction or increase of I^{131} uptake by thyroid metastases, which has been observed (11, 12) following thyroidectomy.

In contrast to the results during the absorption period, we find that during (b), the phase of release of a thyroidectomizing dose, the SRD is higher

in most cases than that delivered during the same interval for a therapeutic dose to the same patient after thyroidecrexis (destruction of the thyroid) (13). This finding is consistent with the following hypothesis: In the presence of the normal thyroid, much of the circulating blood iodine will be removed initially by the gland and then incorporated into thyroglobulin and, in turn, into thyroid hormone. This will eventually be released into the blood and will elevate or maintain the blood I^{131} level for a considerable time. However, following thyroidectomy, there will be no such release of I^{131} into the blood and the radiation will tend to decrease.

If, on the other hand, following thyroidectomy, the total thyroid hormone-synthesizing function of the metastases becomes approximately equal to or greater than that of the non-neoplastic thyroid, it is likely that the blood levels during the period of radioiodine release will be as high, or higher, than during "release" of the thyroidectomizing dose.

One might expect that in such a patient, for succeeding doses, the blood

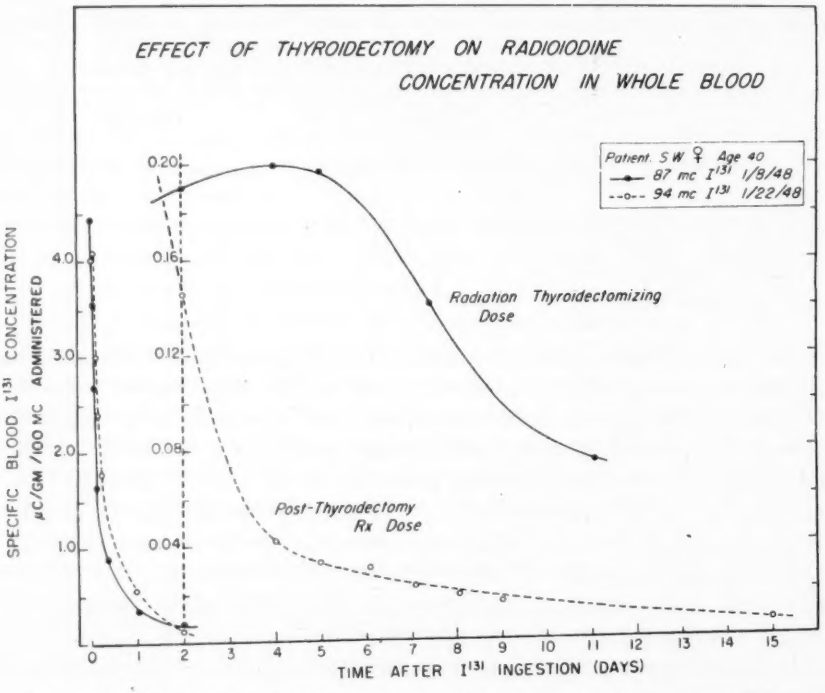
EFFECT OF THYROIDECTOMY ON RADIOIODINE
CONCENTRATION IN WHOLE BLOOD
Patient. S W ♀ Age 40
87 mc I131 1/8/48
94 mc I131 1/22/48
Radiation Thyroidectomizing Dose
Post-Thyroidectomy Rx Dose
SPECIFIC BLOOD I131 CONCENTRATION μC/GM /100 μC ADMINISTERED
TIME AFTER I131 INGESTION (DAYS)

FIG. 2. Specific blood radioiodine concentration curves for thyroidectomizing and post-thyroidectomy doses of I^{131}. Note reversal in relative concentration about two days following ingestion of the isotope.

levels during the release period will vary with the change in the functional status of the thyroid metastases.

The reversal of the relative blood radioiodine concentration after a post-thyroidectomy dose and after a thyroidectomizing dose of I^{131}, which occurs about two days after ingestion of the isotope, is illustrated in Figure 2.

Because of this reversal, we have found no pronounced change in the total SRD delivered during the entire period of irradiation following the thyroidectomizing dose and subsequent doses of I^{131}.

IRRADIATION-PRODUCED RISE IN BLOOD I^{131} CONCENTRATION

Following ingestion of a radiation thyroidectomizing dose of I^{131} to euthyroid patients, a rise in the blood radioiodine concentration has been found (14), usually from four to eight days after administration. This rise has been observed after all such doses, when the blood samples were taken for longer than five days. The magnitude of the rise is correlated with the amount of I^{131} taken up by the thyroid gland. This rise we attribute, in considerable part, to the release of the isotope from the thyroid as a direct result of radiation trauma from the I^{131} concentrated within the gland. The rise has, therefore, been designated as the irradiation-produced rise (IPR).

A rise during this interval has also been observed following about 25 per cent of the therapeutic doses administered to totally thyroidectomized patients with functioning metastases. Figure 1 shows such a rise occurring four days after ingestion of 198 mc. of I^{131} by Patient B.N., who had several functioning metastases but no detectable uptake in the thyroid.

SUMMARY

The radiation delivered to the blood of 23 patients following 53 oral therapeutic doses of I^{131} has been determined from the I^{131} concentration of serial blood samples taken after ingestion of the isotope.

Published results show that the ratio of the average I^{131} concentration in the body to that in whole blood varies with time and equals $\frac{1}{2}$ or less. To give a reasonable upper limit for the radiation received by the blood and by the whole body during I^{131} therapy, this ratio has been assumed to be $\frac{1}{2}$.

Using this assumption in our calculations, we found that the average specific radiation dosage to the blood (REP/100 mc. I^{131} administered) was 52 ± 31. The range was from 20 to 182 REP/100 mc. The maximum total body radiation delivered following a single dose of radioiodine has been calculated to equal 160 REP.

No untoward effects followed the administration of a single therapeutic dose of radioiodine, other than temporary leukopenia. On the other hand, after extensive treatment with several large doses of radioiodine, persistent leukopenia developed in 2 out of 13 patients.

Although the blood I^{131} concentration is lower for the first two days following a radiation thyroidectomizing dose than during the same period following a post-thyroidectomy dose, the reverse is usually observed after the first two days.

A characteristic rise in the blood radioiodine concentration has been observed following thyroidectomizing doses to euthyroid patients and following 25 per cent of the therapeutic doses to thyroidectomized patients with functioning thyroid metastases.

Acknowledgment

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REFERENCES

1. Metzger, F., and Deutsch, M.: The disintegration scheme of I^{131}, *Physical Rev.* **74:** 1640–1648 (Dec. 1) 1948.
2. National Bureau of Standards: Safe Handling of Radioactive Isotopes. Washington, D. C., U. S. Dept. of Commerce, Handbook 42, 1949, p. 6.
3. Bush, F.: The integral dose received from a uniformly distributed radioactive isotope, *Brit. J. Radiol.* **22:** 96–105 (Feb.) 1949.
4. Marinelli, L.; Quimby, E., and Hine, G. J.: Dosage determination with radioactive isotopes, *Am. J. Roentgenol.* **59:** 260–281 (Feb.) 1949.
5. Scott, K. G.; Reavis, J. C.; Saunders, W. W., and White, W. E.: The use of I^{131} red cell plasma ratio as a measure of thyroid function, *Proc. Soc. Exper. Biol. & Med.* **76:** 592–595 (March) 1951.
6. Myant, N. B.; Corbett, B. D.; Honour, A. J., and Pochin, E. E.: Distribution of radioiodide in man, *Clin. Sc.* **9:** 405–419 (Nov.) 1950.
7. Berson, S. A.; Yalow, R. S.; Sorrentino, J., and Roswit, B.: The determination of thyroidal and renal plasma I^{131} clearance rates as a routine diagnostic test of thyroid dysfunction, *J. Clin. Investigation,* **31:** 141–158 (Feb.) 1952.
8. Myant, N. B., and Pochin, E. E.: The metabolism of radiothyroxine in man, *Clin. Sc.* **9:** 421–440 (Nov.) 1950.
9. Marinelli, L., and Hill, R. F.: Radiation dosimetry in the treatment of functional thyroid carcinoma with I^{131}, *Radiology* **55:** 494–502 (Oct.) 1950.
10. Los Alamos Scientific Laboratory: Effects of Atomic Weapons, 1950, p. 342.
11. Seidlin, S. M.; Oshry, E., and Yalow, A. A.: Spontaneous and experimentally induced uptake of radioactive iodine in metastases from thyroid carcinoma: a preliminary report, *J. Clin. Endocrinol.* **8:** 423–432 (June) 1948.
12. Rawson, R. W.; Marinelli, L. D.; Skanse, B. N.; Trunnell, J., and Fluharty, R. G.: The effect of total thyroidectomy on the function of metastatic thyroid cancer, *J. Clin. Endocrinol.* **8:** 826–841 (Oct.) 1948.
13. Winchester, C. F.: Comar, C. L., and Davis, G. K.: Thyroid destruction by I^{131} and replacement therapy, *Science* **110:** 302 (Sept. 23) 1949.
14. Seidlin, S. M.; Yalow, A. A., and Siegel, E.: Irradiation produced rise in blood radioiodine concentration following ingested therapeutic dose for metastatic thyroid carcinoma, *Proc. Soc. Exper. Biol. & Med.* **76:** 711–714 (Apr.) 1951.

THE EFFECT OF PROPYLTHIOURACIL ON THE THYROID UPTAKE OF I^{131} AND THE PLASMA CONVERSION RATIO IN HYPERTHYROIDISM*

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OF THE current antithyroid agents, propylthiouracil is probably the most widely employed in clinical practice. There have been conflicting reports in the literature regarding the mechanism of action of this drug and related compounds, and the effects of the thiourea derivatives on the trapping of iodine and the synthesis of thyroid hormone have not been completely defined. Most of the reported studies have been on experimental animals or tissue studies *in vitro*.

Franklin, Chaikoff, and Lerner (1) found that surviving thyroid slices, incubated in the presence of thiouracil, failed to convert iodide to organically bound iodine, but retained the capacity to concentrate inorganic iodide. In the intact rat thiouracil causes the thyroid gland to enlarge, depresses the thyroid accumulation of radioactive iodine, and inhibits the formation of diiodotyrosine and thyroxine (2, 3, 4). McGinty (5) found that in rats treated with thiouracil, an increased iodine intake caused a proportional increase of thyroid iodine in the form of inorganic iodide. In normal rats the same proportional increase in thyroid iodine occurred, but as organically bound iodine, suggesting that thiouracil blocked hormone synthesis but not the trapping of inorganic iodide. It has been reported that a thiouracil-treated thyroid gland has a greater than normal capacity for concentrating iodide without converting it to the organic form (3–8). In chicks thiouracil promptly produces a block to radioactive iodine collection, but there is a rapid escape from this block when the drug is stopped (9, 10). It has been suggested by some authors (3, 6, 11) that the conflicting views regarding the ability of the thiouracil-treated thyroid gland to take up iodine may be due to variations in the time rela-

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tionship between the administration of radioactive iodine and the subsequent determination of the thyroid uptake. Thus, it has been stated that the thiouracilized thyroid gland can readily take up iodide but is unable to retain it, since the iodide is not converted to organically bound iodine. Therefore one might expect a high uptake shortly after administration of a tracer dose of radioactive iodine, and a subsequent rapid fall to lower levels as the inorganic iodide is released. In general, the experimental studies in animals have suggested that the iodide concentrating capacity of the thyroid is not altered and may be enhanced by the thiourea derivatives; however, the synthesis of thyroid hormone is blocked.

In 1944, Rawson and associates (12) reported that 9 of 10 hyperthyroid patients being prepared for surgery with thiouracil excreted 55 per cent or more of a tracer dose of radioactive iodine in twenty-four hours, and concluded that the thyroid uptake was depressed by thiouracil. Stanley and Astwood (13, 14) found that a single dose of propylthiouracil markedly inhibited the accumulation of I^{131} by the thyroid gland in normal and thyrotoxic human subjects. The accumulated I^{131} was rapidly and almost completely discharged in the propylthiouracil-treated patients but not in the control group, following the administration of thiocyanate. This was interpreted as evidence to support the concept that the accumulated iodine was being held in the propylthiouracil-treated gland as freely diffusible inorganic iodide. This discharge of accumulated thyroid iodine by administration of thiocyanate was also demonstrated in propylthiouracil-treated rats by VanderLaan and VanderLaan (8).

The present study was undertaken in an effort to clarify the effects of propythiouracil on the thyroid collection and rate of organic binding of radioactive iodine in human hyperthyroid subjects.

METHODS

Studies were conducted on 9 hyperthyroid patients, 7 males and 2 females. Eight patients had diffuse toxic goiters and 1 patient had a multinodular toxic gland. The diagnosis of hyperthyroidism was based on clinical and laboratory findings, including the thyroid uptake of I^{131}. None of the patients had received any treatment for hyperthyroidism prior to this study.

The thyroid uptake of radioactive iodine was determined before treatment in each patient, and this served as a control for the subsequent studies. Each patient then received 300 mg. of propylthiouracil daily for a period of from fourteen to sixty days. The thyroid uptake determinations were repeated during this period of treatment, the dose of the drug being administered as usual on the day of the study. Treatment with propylthiouracil was then discontinued, and two to nine days later the thyroid

uptake studies were again repeated. One patient was studied only before and during treatment with the antithyroid drug.

The thyroid uptake of radioactive iodine was determined after administration of an oral tracer dose of 100 microcuries of I^{131}. Radioactivity measurements were made over the thyroid gland and the cup containing the tracer dose, with an RCL model-12 bismuth cathode Geiger-Müller counter, at a distance of 25 cm. In most of the patients counts over the thyroid gland were recorded at one, two, four, eight, twelve, twenty-four and forty-eight hours after administration of the tracer dose. In 2 patients the thyroid uptake of I^{131} was measured only at the twenty-four-hour period at each stage of the study. The thyroid uptake was expressed in per cent of the tracer dose collected by the thyroid gland. In hyperthyroidism this value is greater than 40 per cent in the first twenty-four hours.

In 7 of the 9 patients the total and the protein-bound I^{131} in the plasma were measured twenty-four hours after administration of the tracer dose at each phase of the study. The plasma samples were dried with an infra-red lamp, and the radioactivity measured in an NMC model PC-28 proportional counter. The protein-bound fraction of the plasma was separated by the trichloracetic acid method described by Clark and coworkers (15, 16, 17). The ratio of the protein-bound to the total plasma I^{131} was calculated and expressed as a percentage. Clark has termed this the "conversion ratio." It is an indirect measure of the rate of conversion of inorganic iodide to organically bound iodine by the thyroid gland. Twenty-four hours after administration of a tracer dose, the plasma conversion ratio primarily reflects changes in the level of the protein-bound I^{131}. In hyperthyroidism the conversion ratio at twenty-four hours is greater than 50 per cent.

RESULTS

During the control period the range of maximum thyroid uptake of I^{131} was 60 to 94 per cent, with a mean of 74 per cent (Table 1). Curves for the thyroid uptake of radioactive iodine in the control period showed a rapid increase during the first eight to twelve hours, followed by a plateau, and then a very gradual fall in the succeeding hours.

During administration of propylthiouracil the range of maximum thyroid uptake was 18 to 66 per cent, and the mean for the group was reduced to 38 per cent (Table 1). Only 3 patients had thyroid uptakes greater than 40 per cent while receiving propylthiouracil (G.C., T.Y., and G.Fi.). Clinically, all the patients had shown marked improvement and were at or near the euthyroid state. The curves for thyroid uptake during propylthiouracil administration showed a marked depression at all time intervals.

After stopping propylthiouracil for an average of six days, the range of maximum thyroid uptake was 50 to 88 per cent, and the mean value for the group had returned to the control level of 74 per cent (Table 1). One patient (H.A.) was studied two days and another (R.E.) four days after discontinuing treatment and in both patients the thyroid uptake of I^{131}, although again in the hyperthyroid range, had not yet returned to the control level. In the remainder of the patients the thyroid uptake had

TABLE 1. THYROID UPTAKE OF I^{131} BEFORE, DURING, AND AFTER PROPYLTHIOURACIL TREATMENT

In per cent of oral tracer dose

Patient		Time in hours after tracer dose							Days treated	Days after treatment
		1	2	4	8	12	24	48		
	B*	—	59	79	80	—	94	90		
G. C.	D	40	54	62	67	68	66	64	24	
	A	—	—	86	88	82	80	81		6
	B	—	60	61	70	—	84	66		
G. Fa.	D	9	13	20	23	23	22	24	21	
	A	36	49	70	73	68	69	65		7
	B	37	54	72	76	74	72	56		
T. Y.	D	15	22	34	47	56	57	48	26	
	A	46	61	77	83	82	72	70		6
	B	—	—	—	—	—	62	—		
H. A.	D	4	16	19	23	27	28	31	24	
	A	8	14	30	39	41	50	49		2
	B	6	19	33	37	52	60	48		
P. L.	D	6	14	14	12	12	15	18	33	
	A	—	—	—	—	—	—	—		—
	B	33	41	63	88	75	78	74		
G. H.	D	34	36	33	30	32	37	35	28	
	A	54	62	80	85	80	78	73		7
	B	24	37	49	60	61	70	75		
R. E.	D	21	32	32	24	25	28	27	27	
	A	22	—	44	48	59	61	60		4
	B	—	—	—	—	—	62	—		
G. Fi.	D	—	—	—	—	—	49	—	14	
	A	—	—	—	—	—	71	—		6
	B	—	—	—	—	—	74	—		
K. S.	D	—	—	—	—	—	38	—	60	
	A	—	—	—	—	—	81	—		9
	B	*25*	*45*	*60*	*69*	*66*	*73*	*68*		
Mean	*D*	*18*	*27*	*31*	*32*	*35*	*38*	*35*	*28.5*	
	A	*33*	*46*	*65*	*69*	*69*	*70*	*66*		*6*

* B = before; D = during; A = after.

returned to control hyperthyroid levels six to nine days after discontinuing propylthiouracil.

The per cent reduction in thyroid uptake during treatment was determined for each patient at each time interval studied, and the means calculated (Table 2). The mean per cent reduction in thyroid uptake was less during the first two hours after administration of the tracer dose than in the succeeding hours. There was no significant difference in the mean reduction at four hours when compared to the reduction at eight, twelve, twenty-four and forty-eight hours. The mean reduction in thyroid uptake at twenty-four hours was 49 ± 20.6 per cent.

TABLE 2. REDUCTION IN THYROID UPTAKE OF I^{131} DURING PROPYLTHIOURACIL TREATMENT

In per cent as compared to control values

Patient	Time in hours after tracer dose						
	1	2	4	8	12	24	48
G. C.	—	8	22	16	—	30	29
G. Fa.	—	78	67	67	—	74	64
T. Y.	60	59	53	38	24	21	14
H. A.	—	—	—	—	—	55	—
P. L.	0	26	58	68	77	75	63
G. H.	0	12	48	66	57	53	53
R. E.	12	14	35	60	59	60	64
G. Fi.	—	—	—	—	—	21	—
K. S.	—	—	—	—	—	49	—
Mean	*18*	*33*	*47*	*53*	*54*	*49*	*48*

The mean curves of thyroid uptake of I^{131} before, during, and after propylthiouracil treatment are shown in Figure 1, and are representative of the individual curves. The equation which best fits the observed curves is

$$C(t) = C_m(1 - e^{-kt})$$

wherein $C(t)$ is the per cent thyroid uptake of I^{131} at any time t, C_m is the maximum per cent thyroid uptake, and k is the rate constant. Using the foregoing equation it is possible to analyse the individual curves of thyroid uptake for k, the rate constant, expressed in per cent of the maximum uptake per hour. This equation is essentially the same as that used by Luellen and associates (18) in analyzing the curves of thyroid uptake for normal, hyperthyroid, and hypothyroid patients. During the control period the mean rate constant was 40.7 ± 17.8 per cent per hour (range: 18.2 to 71.4). This is comparable to the value of 40.3 ± 3.7 per cent per

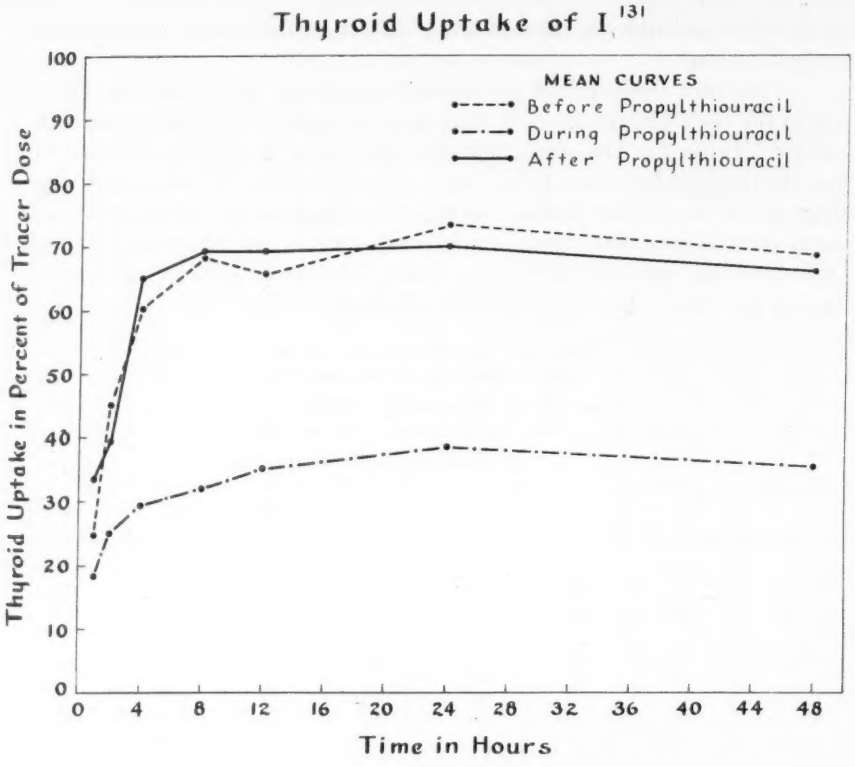

FIG. 1. The mean curves of thyroid uptake of I^{131} for 9 hyperthyroid patients before, during, and two to nine days after treatment with propylthiouracil.

hour, as determined in 15 hyperthyroid patients by Luellen and coworkers (18), and considerably higher than the value of 13.5 ± 0.9 as determined for normal individuals by these same authors. During propylthiouracil administration the mean rate constant was 42.8 ± 21.4 per cent per hour (range: 24.6 to 71.4), and after the drug was discontinued the mean value was 54.9 ± 24.6 per cent per hour (range: 19.8 to 73). There was no significant difference in the mean rate constants before, during, or after propylthiouracil administration.

The values for the plasma conversion ratios are given in Table 3. The mean conversion ratio was 67 per cent during the control period, and in only 1 patient (H.A.) was the ratio less than 50 per cent. During the administration of propylthiouracil there was a 73 ± 19 per cent reduction in the mean conversion ratio. The per cent reduction in the mean conversion ratio at twenty-four hours was significantly greater than that of the

mean thyroid uptake of I^{131} at twenty-four hours ($t=2.708$, $p<0.02$). In none of the patients was the conversion ratio still in the hyperthyroid range during propylthiouracil administration. In 1 patient (T.Y.) the counts for tracer doses administered during and after propylthiouracil were identical and thus the actual counts of the protein-bound I^{131} could be compared directly. In each instance the counts were corrected for background by the appropriate use of plasma blanks. During therapy, there was a 77 per cent decrease in counts at twenty-four hours after the tracer dose. This was comparable to the 73 per cent reduction in the mean conversion ratio for the entire group during propylthiouracil administration.

TABLE 3. PLASMA CONVERSION RATIOS BEFORE, DURING, AND AFTER PROPYLTHIOURACIL

In per cent at 24 hours after I^{131} tracer dose

Patient	Before	During	After	Per cent reduction during propylthiouracil
G. C.	72	41	76	43
G. Fa.	83	18	42	78
T. Y.	79	34	54	57
H. A.	46	5	27	89
P. L.	52	5	—	90
G. H.	66	—	68	—
R. E.	72	13	48	81
Mean	*67*	*19*	*53*	*73*

DISCUSSION

Euthyroidism induced by propylthiouracil is associated with a marked reduction in the quantity of radioactive iodine accumulated by the thyroid gland, to or approaching the level for normal individuals. The curve of I^{131} uptake is otherwise unchanged. There is no early high peak with a subsequent rapid fall, as has been suggested from animal studies. In the present study it has been shown that although the hyperthyroid patient is made clinically euthyroid with propylthiouracil, the rate constant for the thyroid uptake curve is unaltered and remains at the hyperthyroid level. Thus the quantity of radioactive iodine accumulated by the thyroid gland is markedly reduced by this antithyroid drug, but the rate at which it is accumulated is unchanged. On the basis of current physiologic concepts the reduced quantity of thyroid iodine represents mainly a decrease in the organically bound fraction with probably little change in the quant-

ity of inorganic iodide collected by the gland. The rate constant of the uptake curve is most likely a function of the inorganic iodide-collecting capacity of the thyroid gland, which apparently is not affected by propylthiouracil. The persistence of physiologic abnormality is consistent with the anatomic changes produced in the thyroid gland by propylthiouracil. The tissue is not returned to normal, but histologically remains hyperplastic. The plasma conversion ratio is reduced to euthyroid levels and indirectly reflects the decreased formation and release of thyroid hormone by the thyroid gland. The thyroid I^{131} uptake represents both the thyroid collection of inorganic iodide and the accumulation of organically bound iodine, whereas the plasma conversion ratio is related only to the synthesis and release of organically bound iodine. Further evidence that propylthiouracil blocks primarily thyroid hormone synthesis is reflected in the disproportionately greater reduction in the twenty-four-hour plasma conversion ratio as compared to the reduction in the twenty-four-hour thyroid uptake of I^{131} during propylthiouracil administration.

It is apparent from the present study that the maximum thyroid uptake of I^{131} and the plasma conversion ratio are not valid for diagnostic purposes while the patient is receiving propylthiouracil. However, under such circumstances the rate constant for the thyroid uptake curve will still separate the hyperthyroid from the normal patient. Six days after propylthiouracil is stopped the quantity of I^{131} taken up by the thyroid gland and the plasma conversion ratio are once again back at the pretreatment hyperthyroid level, and can be used in the diagnosis of hyperthyroidism.

During the first nine days after the drug is discontinued, there is no evidence of an increased thyroid uptake over the control pretreatment values. The rate constant after discontinuing propylthiouracil is not significantly changed; however, only a small group of patients was studied. The thyroid uptake of I^{131} and the plasma conversion ratio return to hyperthyroid levels several days before a return of clinical thyrotoxicosis becomes apparent.

SUMMARY AND CONCLUSIONS

1. In 9 hyperthyroid patients the thyroid uptake of I^{131} and the plasma I^{131} conversion ratio were studied before, during, and shortly after treatment with propylthiouracil.

2. Prior to treatment the mean maximum I^{131} uptake by the thyroid during the first forty-eight hours after administration of a tracer dose was 74 per cent, and the mean plasma conversion ratio was 67 per cent. Analysis of the thyroid uptake curves revealed a mean rate constant of 40.7 ± 17.8 per cent per hour.

3. In these hyperthyroid patients treated with propylthiouracil for

periods less than two months, the following changes were observed:

a. The quantity of I^{131} accumulated by the thyroid gland was reduced, but the rate constant for the thyroid uptake curve was not significantly changed.

b. The mean curve of I^{131} uptake was reduced by 47 to 54 per cent for all time intervals from four to forty-eight hours after administration of the tracer dose. The reduction was less during the first two hours after the tracer dose. There was, however, no evidence of an early peak in the curve of thyroid uptake, nor of a rapid release of accumulated radioactivity.

c. The plasma conversion ratio was reduced to euthyroid levels. The per cent reduction at twenty-four hours in the mean conversion ratio was significantly greater than the per cent reduction in the mean thyroid uptake of I^{131} at twenty-four hours.

4. When propylthiouracil was given to hyperthyroid patients for less than two months and then stopped, a period of six days was adequate for complete return of the thyroid uptake of I^{131} and the plasma conversion ratio to the pretreatment hyperthyroid levels. This occurred even though the patients remained clinically improved. Analysis of the thyroid uptake curves revealed no increase in either the rate of thyroid uptake or the maximum thyroid accumulation of radioactive iodine over the pretreatment level.

5. The data support the concept that propylthiouracil primarily blocks hormone synthesis by the thyroid gland.

Acknowledgment

The authors are indebted to Mr. Sol Sandhaus for his technical assistance.

REFERENCES

1. Franklin, A. L.; Chaikoff, I. L., and Lerner, S. R.: The influence of goitrogenic substances on the conversion in vitro of inorganic iodide to thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as an indicator, *J. Biol. Chem.* **153:** 151–162 (Apr.) 1944.
2. Franklin, A. L.; Lerner, S. R., and Chaikoff, I. L.: The effect of thiouracil on the formation of thyroxine and diiodotyrosine by the thyroid gland of the rat with radioactive iodine as indicator, *Endocrinology* **34:** 265–268 (Apr.) 1944.
3. Taurog, A.; Chaikoff, I. L., and Feller, D. D.: The mechanism of iodine concentration by the thyroid gland, *J. Biol. Chem.* **171:** 189–201 (Nov.) 1947.
4. Astwood, E. B., and Bissell, A.: Effect of thiouracil on the iodine content of the thyroid gland, *Endocrinology* **34:** 282–296 (Apr.) 1944.
5. McGinty, D. A., and Sharp, E. A.: Effect of iodine intake on thyroid iodine distribution and thyroid weight of rats treated with thiouracil and other goitrogens, (abstract) *Endocrinology* **39:** 74 (July) 1946.
6. VanderLaan, W. P., and Bissell, A.: Effects of propylthiouracil and of potassium

thiocyanate on the uptake of iodine by the thyroid gland of the rat, *Endocrinology* **39:** 157–160 (Aug.) 1946.

7. Astwood, E. B.: Chemotherapy of hyperthyroidism, *Harvey Lect.* **40:** 195–235, 1945.
8. VanderLaan, J. E., and VanderLaan, W. P.: The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate, *Endocrinology* **40:** 403–416 (June) 1947.
9. Larson, R. A.; Keating, F. R., Jr.; Peacock, W., and Rawson, R. W.: A comparison of the effect of thiouracil and of injected thyrotropic hormone on the collection of radioactive iodine and the anatomic changes induced in the thyroid of the chick, *Endocrinology* **36:** 149–159 (Feb.) 1945.
10. Larson, R. A.; Keating, F. R., Jr.; Peacock, W., and Rawson, R. W.: The effect of thiouracil on the collection of radioactive iodine by the thyroid of the chick, *Endocrinology* **36:** 160–169 (Feb.) 1945.
11. McGinty, D. A.; Rawson, R. W.; Fluharty, R. G.; Wilson, M.; Riddell, C., and Yee, H.: The effect of certain goitrogenic drugs on the absorption of radioactive iodine by the thyroid gland, *J. Pharm. & Exper. Therap.* **93:** 246–257 (June) 1948.
12. Rawson, R. W.; Evans, R. D.; Means, J. H.; Peacock, W. C.; Lerman, J., and Cortell, R. E.: The action of thiouracil upon the thyroid gland in Graves' disease, *J. Clin. Endocrinol.* **4:** 1–11 (Jan.) 1944.
13. Stanley, M. M., and Astwood, E. B.: Determination of the relative activities of antithyroid compounds in man using radioactive iodine, *Endocrinology* **41:** 66–84 (July) 1947.
14. Stanley, M. M., and Astwood, E. B.: The accumulation of radioactive iodide by the thyroid gland in normal and thyrotoxic subjects and the effect of thiocyanate on its discharge, *Endocrinology* **42:** 107–123 (Feb.) 1948.
15. Clark, D. E.; Moe, R. H., and Adams, E. E.: The rate of conversion of administered inorganic radioactive iodine into PBI of plasma as an aid in the evaluation of thyroid function, *Surgery* **26:** 331–340 (Sept.) 1949.
16. Sheline, G. E., and Clark, D. E.: Index of thyroid function: Estimation by rate of organic binding of I^{131}, *J. Lab. & Clin. Med.* **36:** 450–454 1950.
17. Sheline, G. E.; Moore, M. C.; Kappas, A., and Clark, D. E.: A correlation between the serum protein-bound iodine and the radioiodine conversion ratio in various thyroid states, *J. Clin. Endocrinol.* **11:** 91–97 (Jan.) 1951.
18. Luellen, T. J.; Keating, F. R., Jr.; Williams, M.; Berkson, J.; Power, M., and McConahey, W.: Relative measurement in vivo of accumulation of radioiodine by the human thyroid gland—comparison with radioactivity in peripheral tissues, *J. Clin. Investigation* **28:** 207–216 (Mar.) 1949.

CALCULATION OF PROTEIN-BOUND IODINE FROM KINETIC DATA

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MOST current methods for the determination of the plasma protein-bound iodine estimate the iodine in the final step by measuring its catalytic effect on the rate of the reaction between ceric ion and arsenious acid. The rate of the reaction is measured photometrically by following the rate of conversion of the yellow ceric ion to the colorless cerous ion. By interspersing suitable standards between the unknown solutions one can usually interpolate the concentration of the unknown from a standard-curve.

Barker *et al.* (1, 2) have proposed that individual samples might contain other substances which could exert inhibitory or catalytic effects; to obviate this possibility they used two aliquots of the final solution. A known amount of iodide was added to the second aliquot on the assumption that extraneous inhibitors or catalysts would exert a similar effect on both the standard and the unknown; thus, each unknown was compared against a second sample containing the unknown plus a standard amount of iodide. The concentration of the unknown was determined from the two sets of data by graphic analysis.

The principle of this method is general. For example, in a photometric procedure let

a = optical density of unknown,
b = optical density of unknown plus standard.

Then,

$$\text{concentration of unknown} = a/(b-a) \times \text{concentration of standard.} \quad \text{(I)}$$

It is the purpose of this paper to present a simple analytic method for the calculation of the protein-bound iodine which offers certain advantages over the graphic method.

EXPERIMENTAL

The method of Barker (2) was followed in detail. The final reaction mixture consisted of 3.0 ml. of the solution to be analyzed, 1.0 ml. of water (or standard sodium iodide containing 0.04 microgram of I), 0.5 ml. of 0.1 normal sodium arsenite, and 1.0 ml. of

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0.02 normal ceric ammonium sulfate. Readings of per cent transmittance were made on a Coleman Jr. spectrophotometer at 440 millimicrons in 19×150 mm. cuvettes. This size cuvette greatly facilitates the rapid and complete mixing of the solution after the addition of ceric sulfate. Readings were made at 0.5 and 12.5 minutes. Since the unknown and standard are run under precisely the same conditions, it was found more convenient to dispense with a constant-temperature water bath and to operate at room temperature. Indeed, this practice is preferable, since the transfer of cuvettes from water bath to spectrophotometer is invariably attended by fluctuations in temperature.

ANALYSIS OF DATA

The data were treated graphically and analytically. Since the reaction is essentially first-order with respect to the concentration of ceric ion, a plot of the logarithm of the optical density of the solution against time will produce a straight line as shown in Figure 1. By the original graphic method (2), readings were taken only at 6 and 12 minutes. Straight lines drawn through these points should intersect at zero time. Experience shows that these lines frequently do not intersect exactly at zero time, with the result that construction of the graph becomes subjective. Probably the chief reason for the lack of intersection at zero time lies in the fact that the final volume of solution (5.5 ml.) is made up of four separate pipettings; moreover, the final readings on more concentrated solutions may show deviation from first-order kinetics.

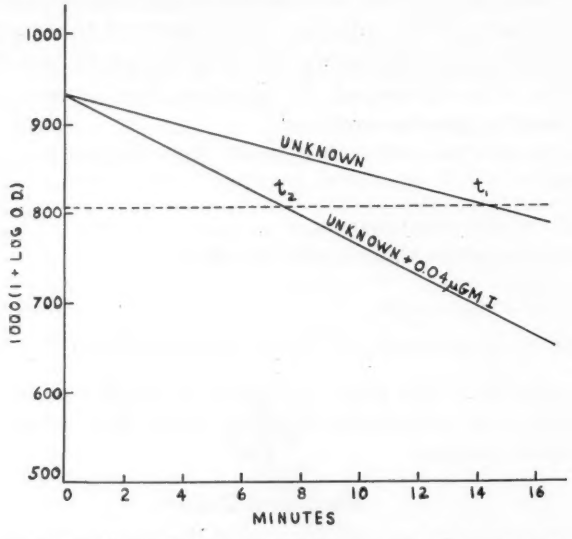

FIG. 1. Graphic analysis for calculation of protein-bound iodine.

$$\text{Micrograms of I in unknown} = \frac{t_2 \times 0.04}{t_1 - t_2}$$

The 0.5-minute reading provides the best base line for further readings. It was found that for all but very high values of iodine, the 6-minute reading could be omitted. Visual inspection of the series is sufficient to detect the presence of a rapid reaction. The second reading at 12.5 minutes is convenient if there are twelve tubes in the series but any suitable time could be chosen without affecting the calculation, provided that both unknown and standard are read at the same time interval.

Let:

$$a = \text{ceric ion concentration at 0.5 minute}$$

and,

$$a - x = \text{ceric ion concentration at 12.5 minutes.}$$

Then,

$$-da/dt = dx/dt = k'(a-x) \tag{II}$$

where k', the first-order reaction velocity constant, includes the concentration of the catalyst.

Hence:

$$k' = \frac{2.3}{t} \log \frac{a}{a-x} \tag{III}$$

and,

$$\log a - \log (a-x) = \frac{12\ k'}{2.3} = k'' \tag{IV}$$

Since the concentration of ceric ion is assumed to be directly proportional to the optical density readings (O.D.), these are substituted in (IV) to give·

$$\log \text{O.D.}_{(0.5)} - \log \text{O.D.}_{(12.5)} = k_c \tag{V}$$

To eliminate negative numbers and decimal fractions, equation (V) is rewritten:

$$1000(1+\log \text{O.D.})_{0.5} - 1000(1+\log \text{O.D.})_{12.5} = k \tag{VI}$$

The value of k is directly proportional to the concentration of catalyst. Values of k are calculated[1] for the unknown (u) and for the unknown plus standard (s). By equation (I):

$$\text{I in unknown} = \frac{k_u}{k_s - k_u} \times \text{I in standard.} \tag{VII}$$

Table 1 illustrates the method of calculation, using representative data for low, normal and high PBI values. Readings were taken in per cent

[1] It is convenient to prepare a table covering the range from 10 to 70 per cent transmittance in steps of 0.1 per cent to give directly values for the function: 1000(1+log O.D.), where O.D. = 2 − log %T.

transmittance and converted to the values of the function: 1000(1+log O.D.). Values for k were calculated according to equation (VI). Values for $(k_s - k_u)$ were obtained and the micrograms of iodine in the unknown then calculated according to equation (VII). Correction for the blank

TABLE 1. CALCULATION OF PROTEIN-BOUND IODINE

Sample		% Transmittance		1000 (1+log O.D.)		k	$(k_s - k_u)$
		0.5	12.5	0.5	12.5		
EP	u	12.8	16.7	951	891	60	
	s	13.0	27.0	948	755	193	133
AR	u	13.8	21.4	935	826	109	
	s	13.8	32.2	935	692	243	134
	u	13.6	21.4	938	826	112	
	s	13.5	31.6	939	699	240	128
JL	u	13.8	30.6	934	711	223	
	s	13.8	41.7	934	580	354	131
Blank	u	13.8	16.3	934	896	38	
	s	13.4	25.5	941	773	168	130
	u	13.7	16.5	936	894	42	
	s	13.6	25.9	938	768	170	128

Sample	μG n. I/tube*	−blank	×700/3
EP	0.0180	0.0056	1.3 μGm. %
AR	0.0326	0.0202	4.7 μGm. %
	0.0350	0.0226	5.3 μGm. %
JL	0.0681	0.0557	13.0 μGm. %
Blank	0.0117		
	0.0131		

* 60/133×0.04=0.0180, etc.

and multiplication by the dilution factor gives the value of protein-bound iodine in units of micrograms per 100 ml. of plasma.

DISCUSSION

The quantity $(k_s - k_u)$ should be relatively constant in any given series of determinations if there are no interfering substances present, since its value represents the increase in the first-order constant due to a fixed amount of iodine (0.04 microgram in the present procedure). The value fluctuates slightly from day to day, depending on the room temperature. A persistent trend toward lower values would result from deterioration of the standard. In a series of 150 determinations in this laboratory the value of the quantity $(k_s - k_u)$ showed an average deviation of ± 14 per cent. Since the temperature coefficient of the reaction was found to be approxi-

mately 1.5, this amount of variation could be caused by an average deviation in room temperature of $\pm 3°$ C. Other factors probably contribute to this variation besides temperature but it is possible, from the analytic data, to detect any specimen which deviates appreciably from normal.

In a consecutive series, 50 samples of plasma were analyzed in duplicate for protein-bound iodine by the alkali incineration technique (2) with subsequent analysis of the data as just described. The average difference between the duplicate determinations was 0.5 microgram per cent (range 0.1 to 1.2 microgram per cent); consequently, the method is considered to be a reliable diagnostic aid in thyroid disease.

SUMMARY

An analytic method is presented for the calculation of plasma protein-bound iodine from kinetic data based on the catalyzed reaction between ceric sulfate and arsenious acid. The method is simple, rapid and objective and offers several advantages over graphic methods.

REFERENCES

1. Barker, S. B., and Humphrey, M. J.: Clinical determination of protein-bound iodine in plasma, *J. Clin. Endocrinol.* **10:** 1136–1141, 1950.
2. Barker, S. B.; Humphrey, M. J., and Soley, M. H.: The clinical determination of protein-bound iodine, *J. Clin. Investigation* **30:** 55–62, 1951.

TEMPORARY HYPOPARATHYROIDISM FOLLOWING RADIOACTIVE IODINE TREATMENT FOR THYROTOXICOSIS

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ALTHOUGH radioactive iodine, I^{131}, has been used successfully in the treatment of thyrotoxicosis since 1943, there have been no published reports of hypoparathyroidism occurring as a complication. We have recently observed temporary hypoparathyroidism in a 14-year-old boy, seventy-four days following a therapeutic dose of I^{131}.

CASE HISTORY

The patient was seen first in May 1950, with a three-year history of tremor, nervousness, and increased sweating. Heat tolerance was normal, as were appetite and weight gain.

The thyroid gland was grossly enlarged, the right lobe measuring 5 by 3 cm., the left lobe 4 by 3 cm., and the isthmus 3 by 3 cm. The gland was firm, with no definite nodules; there was no bruit or thrill. The heart was not enlarged; the rate was 110 and regular, and there were no murmurs. The systolic blood pressure was 110 and the diastolic 60 mm. of mercury. There was no exophthalmos. Several small anterior cervical lymph glands were palpable. The basal metabolic rate was +30 per cent, and the blood cholesterol concentration, 138 mg. per cent. Biopsy of a cervical node showed only mild inflammatory reaction.

The patient received a tracer dose of 50 microcuries of I^{131} on May 22, 1950. The uptake measured over the thyroid was 80 per cent and the urinary excretion in forty-eight hours was 8 per cent. He received 4.0 millicuries of I^{131} on May 25, 1950; the 48-hour urinary excretion was 17 per cent of the therapeutic dose.

There was no significant change until August 2, ten weeks later. He had then gained six pounds and was symptomatically better; the thyroid was about one half its former size. Since the boy still seemed slightly thyrotoxic, however, another tracer dose of 50 microcuries of I^{131} was given on August 2. This revealed 70 per cent uptake in the thyroid. It was therefore planned to give a second therapeutic dose.

Fortunately, the boy went away to camp a few days later and treatment was postponed. On August 6, he began having muscle spasms in his arms each morning for a few minutes. On August 13, the spasms became more severe and frequent and he was taken from camp to a local hospital. The diagnosis of tetany with carpopedal spasm was obvious. No blood samples were taken but intravenous calcium gave immediate relief of the symptoms. He was given some calcium tablets and was seen by the writer the next afternoon. There had been no further spasm.

The Chvostek and Trousseau signs were not present. The thyroid was slightly smaller than when he was last examined, the pulse rate was 80, and the blood pressure, 90/70.

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During the next month, blood studies gave the following results:

Date	Calcium (mg. %)	Phosphorus (mg. %)	Acid phosphatase (Bodansky units)	Basic phosphatase (Bodansky units)
Aug. 14	7.8	6.9	2.2	7.5
Aug. 15	6.3	6.7		
Aug. 18	6.0	8.0		
Aug. 21	6.5	9.8		
Aug. 28	6.0	6.4		
Sept. 5	7.0	6.0		
Sept. 11	8.1	5.2		
Sept. 18	8.8	4.5		
Sept. 23	8.5	6.8		

There were no further symptoms of tetany but on August 28 a blood pressure cuff applied for two minutes at 100 mm. pressure produced definite carpopedal spasm. This spasm was reproduced several times during the next hour. Repeated subsequent similar examinations produced no spasm. As the data show, the blood levels of calcium and phosphorus had returned to normal six weeks after the onset of the tetany.

At that time there was some increase in tremor and the thyroid had increased to about one half of its pretreatment size. The basal metabolic rate on September 30 was +11 per cent, with a resting pulse rate of 94.

Since further radiation therapy or surgical intervention to control the thyrotoxicosis seemed hazardous, propylthiouracil in a dosage of 150 mg. daily was used, beginning on September 30. Some clinical improvement resulted, but on January 2, 1951, the white blood cell count dropped to 4,000 and the drug was discontinued. It was started again on February 19, in a dosage of 300 mg. daily, and was continued until the patient was last seen on July 16, 1951, with no further leukopenia. At that time, the thyroid had not changed significantly in size and there were still signs of mild thyrotoxicosis.

DISCUSSION

It seems likely that the temporary hypoparathyroidism in this case was due to the treatment dose of I^{131}. The mechanism of the production of this radiation lesion is not clear. It has been assumed that the major tissue effects of I^{131} in the thyroid are due to 0.6 mev. beta radiation. One must hypothesize that in order to produce similar lesions in the parathyroids, all four of these glands were surrounded by thyroid tissue, since the effective ionization path of beta radiation is limited to a few millimeters. There are several reports (1, 2) of one parathyroid gland being completely surrounded by thyroid tissue and of single glands occasionally found in grooves of thyroid tissue or carried beneath the thyroid capsule by blood vessels. The 0.37 mev. gamma radiation has a longer path but the degree of tissue ionization is much less. It is possible also that the parathyroid

hypofunction may have been secondary to interference with the blood supply. Since the syndrome did not occur until ten weeks following treatment, it seems unlikely that postradiation edema was a factor.

Surgical exploration in this case to determine the exact location of the parathyroids and the nature of the radiation lesion would have been most informative. However, it seems likely that the parathyroid glands would have been small, difficult to identify, or even buried in the thyroid gland. Furthermore, even minor manipulation might have produced further and irreversible hypoparathyroidism.

As yet, there have been no recorded reports of parathyroid injury in humans following administration of I^{131}. Even the enormous doses used for thyroid carcinoma, which may depress bone marrow and ovarian function, have not produced hypoparathyroidism (3). In certain strains of mice, Gorbman (4) has reported complete destruction of the parathyroids by less than thyroid lethal doses of I^{131}.

CONCLUSION

A case of temporary hypoparathyroidism occurring in a 14-year-old boy, ten weeks following the administration of 4.0 millicuries of I^{131} for thyrotoxicosis, is presented. Judging from the experiences reported to date in the literature, this complication will probably be rather rare but the possibility of its occurrence should be kept in mind.

REFERENCES

1. Gilmour, J. R.: The gross anatomy of the parathyroid glands, *J. Path. & Bact.* **46:** 133–149 (Jan.) 1938.
2. Cope, O.: The surgery of hyperparathyroidism; occurrence of parathyroids in anterior mediastinum and division of operation into 2 stages, *Ann. Surg.* **114:** 706–733 (Oct.) 1941.
3. Dobyns, B. M., and Maloof, F.: Study and treatment of 119 cases of carcinoma of the thyroid with radioactive iodine, *J. Clin. Endocrinol.* **11:** 1323–1360, (Nov.) 1951.
4. Gorbman, A.: Functional and structural changes consequent to high dosages of radioactive iodine, *J. Clin. Endocrinol.* **10:** 1177–1191 (Oct.) 1950.

PERIODIC PARALYSIS ASSOCIATED WITH THYROTOXICOSIS: A CASE REPORT*

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PERIODIC paralysis is a rare disease characterized by recurrent attacks of flaccid paralysis in the absence of any gross involvement of the central nervous system. The attacks usually occur at night, frequently following a high carbohydrate meal. They last from a few hours to a few days and subside, leaving no residual muscle dysfunction.

The disease may appear as a familial trait or may occur sporadically with a negative family background. Talbott (1), in his exhaustive review of the literature on the subject through 1941, found that 18 per cent of the cases reported were of the sporadic type. He also noted that the sporadic type occurred almost exclusively in young males, whereas in the familial type the ratio of males to females was 3:1.

Shinosaki (2), in 1926, first noted the association of periodic paralysis with thyroid disease. He reported a series of 24 cases, 15 of which manifested the clinical characteristics of thyrotoxicosis with associated periodic paralysis. Since this report, an additional 19 cases have appeared in the literature (3–8).

We wish to report in this paper, an additional case occurring in a young Italian male with a negative family history and the clinical characteristics of both thyrotoxicosis and periodic paralysis.

CASE HISTORY

J. C., a 27-year-old white male of Italian descent was first admitted to the Brooklyn Veterans Administration Hospital on October 21, 1950, complaining of tremors and a weight loss of 40 pounds during the six months prior to admission.

Onset of symptoms actually dated back to 1945, when the patient first noted increasing nervousness and perspiration. In spite of their persistence, these symptoms were not incapacitating. In 1948, prominence of the left eye was noted. During this same year the patient had the first of recurrent attacks of paralysis of his lower extremities. These attacks began as a feeling of numbness in the lower limbs, usually occurring at bedtime and following an evening meal high in carbohydrate content. There would be progression to complete paralysis by morning. This paralysis would persist for another

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four or five hours and then gradually subside, with a return to completely normal function. Between attacks there would be no residual weakness of the extremities. Since 1948, there had been three or four such episodes annually.

During 1949 and 1950 the symptoms progressed rapidly. The patient had to leave his job and seek medical attention. His appetite increased tremendously and yet progressive weight loss was noted. When considerable elevation of the BMR was noted, hospitalization was advised.

The family history was noncontributory so far as similar symptomatology was concerned.

Physical examination revealed a well developed, well nourished, apprehensive white male. Lid lag and bilateral exophthalmos, more marked on the left, were apparent. A diffuse, soft, uniform enlargement of both lobes of the thyroid gland was palpable; there was no bruit. Examination of the chest and lungs revealed nothing abnormal. The heart was not enlarged clinically. B.P. was 130/86 and the pulse rate, 128. The cardiac rhythm was regular. A short systolic murmur was heard at the apex and was not transmitted to other areas. Upon abdominal, genital, and rectal examination the findings were within normal limits. The extremities showed both fine and coarse tremors of the hands and fingers. Neurologic examination revealed an intact sensorium. Cranial nerves were normal. Sensation, to light, touch and pain, was normal. Muscle power and tone were normal, as were the deep and superficial reflexes. There were no abnormal reflexes.

The patient's skin was warm and moist, and radiated heat.

X-ray examination showed a normal chest. Blood sugar concentration was 100 mg. per cent. Urinalysis, the serologic test for syphilis, and a hemogram gave normal results.

On admission, the patient was placed at bed rest with no specific therapy and repeated BMR values were obtained. These were +44, +62, and +39 per cent, respectively. On this regimen for approximately two weeks, the patient gained five pounds and maintained it. On November 8, 1950, the administration of 5-iodo-thiouracil, 300 mg. daily, was started. Symptomatically he improved. The tachycardia persisted, however, and his weight remained stationary. The daily dosage of the drug was increased to 400 mg. and on this dose the patient's weight increased and the pulse rate dropped. The BMR also dropped to +16 per cent and the patient was considered to be ready for thyroidectomy.

Thyroid surgery had to be postponed when an infected pilonidal cyst, developed which required incision and drainage. Subsequent to this procedure the BMR rose to +49 per cent. Antithyroid drugs were continued and by December 21, 1950, the BMR had dropped to +21 per cent; it remained at this level.

The patient was sent home on a leave of absence for the Christmas holidays, prior to the performance of a thyroidectomy. On the morning of December 23, he returned to the hospital with complete paralysis of his lower extremities. On the previous night, following a heavy spaghetti dinner, he noted "shooting pains" in his legs. At 2 A.M. on the 23rd, he awoke unable to move his lower extremities. This state persisted for a longer period of time than any of his previous attacks and the patient was brought back to the hospital.

Neurologic examination, repeated at the time of attack, differed from the initial examination in that the patient was unable to move his lower limbs voluntarily and there was moderate depression of the deep tendon reflexes. Otherwise the examination showed the nervous system to be within the physiologic limits of normal.

The blood concentration of sugar and of serum Ca and P were all within normal range; that of serum K was 2.5 mEq./L. A spinal tap revealed clear colorless fluid with normal pressure and dynamics. Chemical analyses of the spinal fluid also gave normal results.

An electrocardiogram revealed lowered T waves with ST segment depression, compatible with abnormalities in serum electrolyte levels. Previous and subsequent electrocardiograms showed nothing abnormal (Fig. 1).

The attack subsided spontaneously four hours after admission. Three subsequent determinations of serum K were 3.9, 4.0 and 3.9 mEq./L., respectively. The lowered serum K and the EKG changes, in addition to the clinical course, were regarded as inescapable evidence for a diagnosis of periodic paralysis of the sporadic type, associated with thyrotoxicosis.

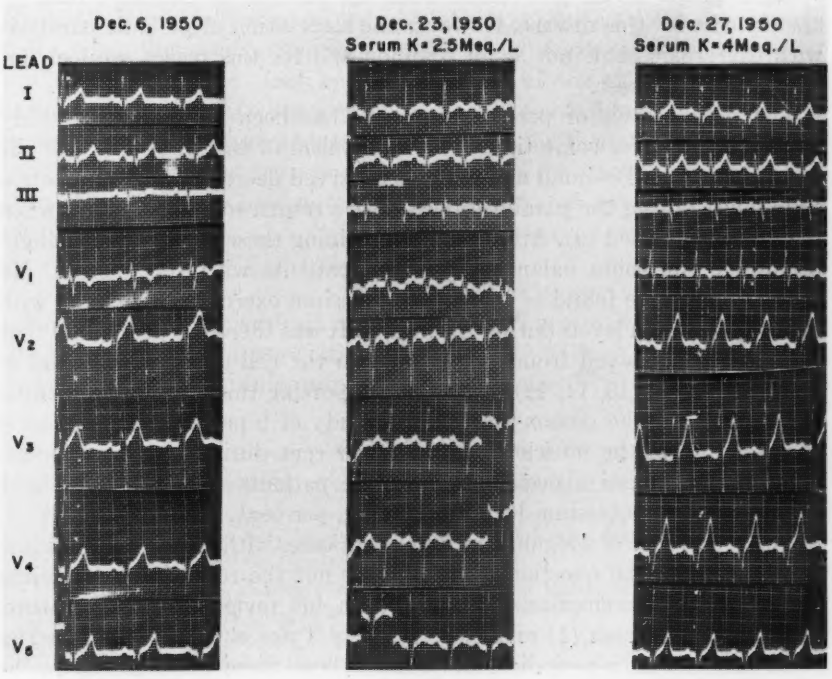

FIGURE 1

Shortly after this episode, the thyrotoxicosis was again considered adequately controlled for surgical intervention and on December 29, 1950, a subtotal thyroidectomy was performed. The postoperative course was uneventful and the patient was transferred back to the Medical Service for further studies.

Attempts were made to precipitate paralytic seizures, using oral and intravenous glucose, glucose and adrenalin, rice diet with adrenalin, and high carbohydrate meals of the type that had previously produced attacks. All were equally ineffective in producing any change in muscle function following thyroidectomy and it was felt that the operation had beneficially affected the tendency toward these attacks.

The patient was then discharged from the hospital. He returned to work as a truck driver. It is now ten months since the thyroidectomy and he is gaining weight and is

feeling very well. There has been no recurrence of paralysis, in spite of high carbohydrate intake.

DISCUSSION

This case is a typical example of the clinical entity called periodic paralysis. The recurrent episodes of flaccid paralysis, absence of significant neurologic findings, spontaneous recovery, and absence of residua in the symptom-free periods, coupled with the electrocardiographic changes and the changes in the serum potassium levels are all well documented findings associated with this disease. However the association of periodic paralysis with thyrotoxicosis is not at all common and for this reason we feel this case to be of interest.

The pathogenesis of periodic paralysis has been a subject for study and speculation for some time. The mechanism of this disease is still not known. In 1934, Biemond and Daniels observed decreases in serum potassium levels during the paralytic state, with a return to normal levels when the attack subsided (9). Attempts at explaining these electrolytic changes prompted potassium balance studies on patients with this disease. No evidence could be found of increased potassium excretion associated with the drop in serum levels during the attack. It was therefore postulated that the potassium moved from the serum into the cell during the period of clinical activity (10, 11, 12). Evidence supporting this idea was presented by Jantz (13), who demonstrated, in a study of 9 patients, a mean level of potassium in the muscle of 710 mg. per cent during the paralytic attack. When remission occurred, the same patients were found to have average muscle potassium levels of 360 mg. per cent.

The occurrence of a significant number of cases without hypopotassemia, however, poses the question of whether or not the role of the potassium ion has been overemphasized. Talbott, in his review of the literature, mentions these cases (1) and more recently Tyler *et al.* reported a series of 33 patients with periodic paralysis in whom there was no drop in the serum potassium levels (14).

The clinical course of the disease, with intermittent episodes of paralysis followed by completely symptom-free periods, suggests an abnormally delicate contractile mechanism which is disturbed intermittently by factors which have no effect on the normal neuromuscular apparatus. These disturbing influences are often extrinsic and include such factors as increased carbohydrate intake, insulin, DCA, and adrenalin. The manner in which these stimuli operate to disrupt the contractile mechanism, when discovered, will probably provide full understanding of periodic paralysis.

There is some evidence to suggest that the chain of events leading to the nonfunctioning of a muscle begins with disruption of ionic equilibrium

which, in turn, affects the other reactions involved in muscular contraction (15, 16, 17). However, it is beyond the scope of this report to discuss this hypothesis in any detail.

What is the relationship between periodic paralysis and thyrotoxicosis? We rarely see these entities associated, in spite of the frequency of hyperthyroidism. Obviously, overactivity of the thyroid gland alone does not initiate the events leading to the paralytic state. We must, therefore assume a separate subclinical abnormality in the contractile apparatus which is aggravated in some way by the addition of the hyperthyroid state. When this occurs, the right combination of external stimuli results in a paralytic seizure. Just how overactivity of the thyroid brings this latent tendency to the foreground is not known. When, however, the hyperthyroidism is corrected, the patient loses the tendency toward paralytic seizures. This has been observed by us and by others who noted that when these patients become euthyroid, attacks of paralysis cannot be precipitated (4, 5, 8).

SUMMARY

A case of periodic paralysis associated with thyrotoxicosis is presented. The literature on the association of these two diseases is reviewed and possible pathogenic mechanisms are briefly discussed.

REFERENCES

1. Talbott, J. A.: periodic paralysis: clinical syndrome, *Medicine* **20:** 85–143 (Feb.) 1941.
2. Shinosaki, T.: Klinische Studien über die periodische Extremitätenlähmung, *Ztschr. f. d. ges Neurol. u. Psychiat.* **100:** 564–611. 1926.
3. Tsuji, K.: Über die Pathogenese der paroxysmalen Extremitätenlähmung, *Klin. Wchnschr.* **18:** 320–324 (March 4) 1939.
4. Dunlap, H. F., and Kepler, E. J.: Syndrome resembling familial periodic paralysis occurring in the course of exophthalmic goiter, *Endocrinology* **15:** 541–546 (Nov.-Dec.) 1931.
5. Hildebrand, A. G., and Kepler, E. J.: Familial periodic paralysis associated with exophthalmic goiter, *J. Nerv. & Ment. Dis.* **94:** 713–721 (Dec.) 1941.
6. Morrison, S., and Levy, M.: Thyroid factor in familial periodic paralysis: report of a case, *Arch. Neurol. & Psychiat.* **28:** 386–393 (Aug.) 1932.
7. Mora, J. M.: Periodic paralysis occurring in the course of exophthalmic goiter, *Endocrinology* **16:** 407–408 (July-Aug.) 1932.
8. Seed, L.: Periodic paralysis in a patient with exophthalmic goiter controlled by 6-propylthiouracil, *West J. Surg.* **55:** 640–646 (Dec.) 1947.
9. Biemond, A., and Daniels, A. P.: Familial periodic paralysis and its transition into spinal muscular atrophy, *Brain* **57:** 91–108 (June) 1934.
10. Gammon, G. D.: Relationship of potassium to familial periodic paralysis, *Proc. Soc. Exper. Biol. & Med.* **38:** 922–924 (June) 1938).

11. Pudenz, R. H.; McIntosh, J. F., and McEachern, D.: Role of potassium in familial periodic paralysis, *J.A.M.A.* **111:** 2253–2258 (Dec. 17) 1938.
12. Ferrebee, J. W.; Gerity, M. K.; Atchley, D. W., and Loeb, R. F.: Behavior of electrolytes in familial periodic paralysis, *Arch. Neurol & Psychiat.* **44:** 830–840 (Oct.) 1940.
13. Jantz, H.; Stoffwechseluntersuchungen bei paroxysmaler Lähmung, *Nervenartz* **18:** 360–378 (Aug.) 1947.
14. Tyler, F. H.; Stephens, F. E.; Gunn, F. D., and Perkoff, G. T.; Studies in disorder of muscle. VII. Clinical manifestations and inheritance of a type of periodic paralysis without hypopotassemia, *J. Clin. Investigation* **30:** 492–502 (May) 1951.
15. Szent-Györgyi, A.: Attacks on muscle, *Science* **110:** 411–413 (Oct. 21) 1949.
16. Best, C. H., and Taylor, N. B.: Physiological Basis of Medical Practice, ed. 3. Baltimore, Williams and Wilkins Company, 1943, pp. 1030–1036.
17. Walker, S. M.: Action potentials in rat muscle with twitch tension potentiated by potassium chloride treatment, adrenalectomy, tetanus and treppe, *Am. J. Physiol.* **154:** 63–72 (July) 1948.

CONGENITAL TESTICULAR DEFICIENCY. I. ABSENCE OF SPERMATOGONIA, SERTOLI AND LEYDIG CELLS AS A CAUSE OF EUNUCHOIDISM WITH CRYPTORCHIDISM

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DEVELOPMENTAL and constitutional factors are among those most frequently considered in the evaluation of male hypogonadism of obscure origin. The concept of constitutional factors as a cause of primary gonadal deficiency is vague, ill defined, and not readily documented. By contrast, developmental defects as a cause of sex gland incompetence can usually be recognized without much difficulty. This applies not only to the grossly recognizable rare cases of gonadal agenesis but also to those instances requiring histopathologic analysis of the testis. The object of the present communication is to report an instance of eunuchoidism due to a congenital testicular defect and to draw attention to the histologic criteria for its recognition despite the presence of cryptorchidism.

CASE REPORT

The patient was a 23-year-old native of Puerto Rico who came to the United States three years previously. He was one of 11 siblings, 4 of whom were females, whose ages ranged between 11 and 30 years. One brother, 26 years of age, had been institutionalized in Puerto Rico on three occasions because of a paranoid type of schizophrenia; he was apparently a sexually normal male whose IQ at the age of 20 years was 65.[1] Four siblings were married and 3 had children. The remaining siblings were healthy and unmarried. The parental relationship was not consanguineous.

The patient attended school from the ages of 10 to 17 years, at which time he had reached the seventh grade. He applied for admission to the hospital because of recurrent right lumbar pain for which no cause was discovered. Except for pneumonia in childhood, there were no significant previous illnesses. There was no history of malnutrition or of disease, injury, operation or irradiation of or near the gonads. Upon further questioning he complained of a general lack of strength and was unhappy about his lack of masculinity. He had never shaved nor had he had any penile erections, nocturnal emissions or erotic desires. An inability to distinguish odors and aromas was present but he claimed normal taste sensation.

Upon examination he was found to measure $69\frac{1}{4}$ inches in height with a finger-to-finger arm span of $71\frac{3}{4}$ inches. The distance from the floor to the symphysis pubis was $37\frac{1}{4}$ inches and that from the symphysis pubis to the vertex was 32 inches. He weighed

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[1] Information supplied through the courtesy of R. Fernandez Marina, M.D., Medical Director, Insular Hospital of Psychiatry, Rio Piedras, Puerto Rico.

144 pounds and presented a typical eunuchoid appearance as illustrated in Figures 1 and 2. The posture was poor, with a characteristic rounding of the back. The hips were wide and the fingers were long and gracile. The skin was soft and silky and the cranial hair was abundant. There was no recession of the hair line in the temporal regions. Hair was absent on the face, body and chest and was present in scant amounts in the axillae and in the pubic region, where it had a feminine type of distribution. There was no enlargement of the mammary tissue. The penis was infantile, measuring $1\frac{1}{4}$ cm. in length, and the urinary meatus was in the normal position. The scrotum was small and rudimentary in type. It was flush with the perineum and no testes could be palpated within the scrotum or in the inguinal regions. There was no hernia. The prostate could not be palpated. The laryngeal cartilages were small and the voice was high in pitch. There was no genu valgum or pes planus. There were no abnormal neurologic findings. There was no evidence of retinal disease and color vision was normal. Intranasal examination revealed evidence of chronic pansinusitis, which accounted for the anosmia.

Roentgen examination of the spine revealed a moderate degree of osteoporosis, particularly of the lumbar vertebrae. The secondary centers of ossification for the dorsal vertebral bodies (annular epiphyses) were unfused. The sacroiliac joints bilaterally were indistinct but this was regarded as being related to the osteoporosis. The epiphyses of the long bones were unfused. The sella turcica was normal in size and configuration. The bones of the calvarium were normal and there was no evidence of increased intracranial pressure. The basal metabolic rate was −8 per cent. The hemoglobin was 14 grams, the leukocytes numbered 6,900 per cu.mm. and the differential leukocyte count was within normal limits. Blood urea nitrogen concentration was 15, cholesterol 240, calcium 10.6, phosphorus 4.2, and sugar 85 mg. per 100 cc. The oral glucose tolerance curve and the erythrocyte sedimentation rate were normal. Urinalysis gave normal findings. The Wassermann reaction was negative. Intravenous pyelography showed no abnormality of the genito-urinary tract.

The assay for urinary gonadotropins gave negative results at 30 mouse uterine units per twenty-four hours on two separate occasions. The method employed was essentially that of Zondek (1) as modified by Klinefelter, Albright and Griswold (2). In our laboratory, with certain additional minor modifications of technic, the normal range is between 5 and 45 mouse uterine units per twenty-four hours. Therefore, the urinary gonadotropin titer of this patient was either normal or subnormal.

The urinary excretion of neutral 17-ketosteroids was reduced to 3.2 mg. per twenty-four hours. The 24-hour urine contained 0.63 mg. of 11-oxygenated steroids, a value which in our laboratory is within the normal range. Seminal fluid could not be obtained for examination.

Psychometric examination revealed the IQ to be 73 by the Full Scale Wechsler-Bellevue technique. The general intelligence was, therefore, in the region between mental deficiency and dull average. However, his IQ on the Verbal Scale was 84, suggesting the absence of true mental deficiency. Further investigation with the aid of the Rorschach, Word Association and Bender tests disclosed the presence of overwhelming distortion of judgment and emotional infantilism during periods of excitement. Confused sexual identification, strong body consciousness and feelings of genital inadequacy were also uncovered. The psychologic data, including the approximately 20 per cent deterioration of the intelligence level, were interpreted as suggestive of a psychiatric disorder.

In an effort to establish the presence or absence of testicular tissue surgical exploration was performed. Through a right lower rectus incision the pertioneal cavity was entered and the pelvic peritoneum explored. A normal vas was seen on each side extend-

FIGS. 1 and 2. Typical eunuchoid appearance of patient, showing increased arm span over height, sparse pubic and axillary hair, very small penis and scrotum,lack of recession of temporal hair line, wide effeminate hips and dorsal kyphosis.

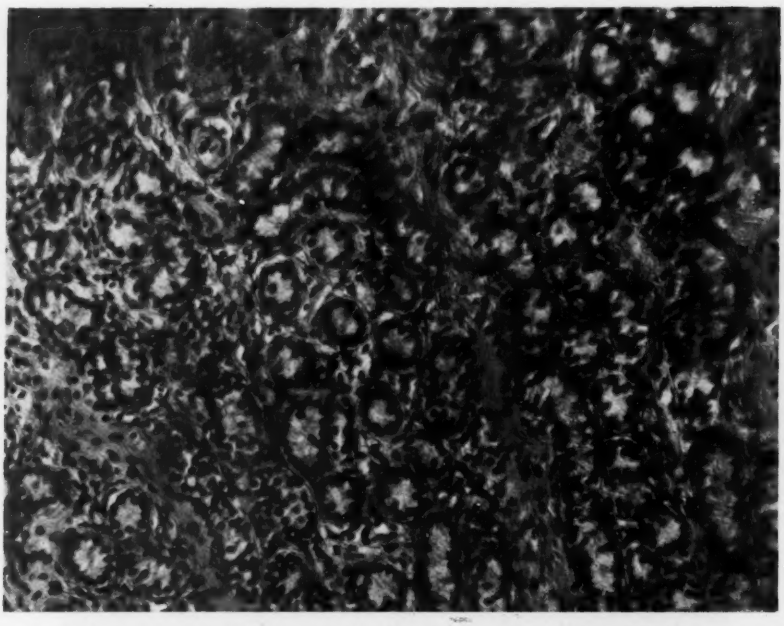

FIG. 3. Photomicrograph of testicular biopsy specimen, low power, showing very small, solid tubules lined by a single layer of undifferentiated cells (H. & E. ×150).

ing from the area of the uretero-vesical junction to the internal ring. Through an oblique right inguinal incision the right inguinal canal was explored. No spermatic cord constituents were found within the canal except at its superior extremity near the internal ring, where a very small pink nodule was present. This measured 5 mm. in diameter and was removed for microscopic study. The other inguinal canal was not explored.

Histologic examination revealed the presence of very small immature tubules with an average diameter of 40 microns and without lumens (Figs. 3 and 4). Distributed along the basement membrane in a single layer was a uniform type of undifferentiated cell characterized by a round or oval nucleus with fine and coarse chromatin granules and a distinct nuclear membrane. The cytoplasm was not well demarcated and projected centrally as a syncytial mass. It was irregularly traversed by delicate fibrillar strands. Spermatogonia could not be definitely identified and Sertoli cells were absent. The basement membrane and tunica propria were neither thickened nor fibrotic. There was no increase or fibrosis of the intertubular connective tissue. The latter was fairly cellular but contained no Leydig or inflammatory cells.

DISCUSSION

A clear understanding of the developmental histology of the normal testis from the fetal through the preadolescent period is essential for the recognition and interpretation of congenital testicular lesions. Pertinent observations on the microscopic appearance of the developing testis have

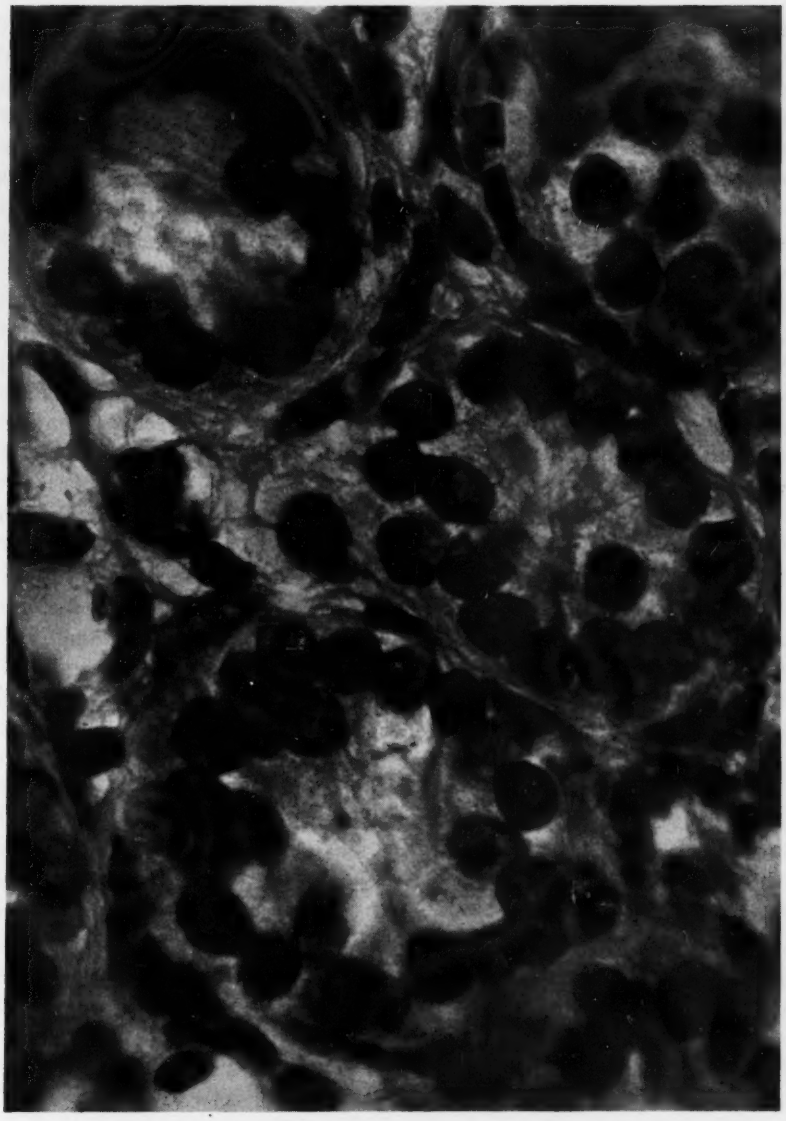

FIG. 4. Photomicrograph of testicular biopsy specimen, high power, showing the nuclear and cytoplasmic details of the undifferentiated cells. Spermatogonia, Sertoli and Leydig cells are absent, basement membrane is not thickened, and there is no interstitial fibrosis (H. & E. ×1,000).

been recorded by Wangensteen (3), Cooper (4) and Nelson (5). Sniffen (6) has recently published a comprehensive report with detailed cytologic studies. Briefly summarized, it may be stated that spermatogonia and undifferentiated cells are present in testicular tubules from fetal life until puberty. Interstitial cells of Leydig are prominent antenatally and disappear a few months after birth. The advent of adolescence is characterized by a marked increase in tubular diameter, the initiation of spermatogenesis, full differentiation of Sertoli cells from the undifferentiated cells and the reappearance of Leydig cells.

Of utmost importance in the evaluation of prepuberal testicular histology is the fact that spermatogonia are always present and readily identifiable under normal conditions. They may diminish in number or disappear as a result of noxious influences such as local trauma, inflammation, neoplasia, vascular compromise or exposure to radioactive agents. A reduction in the number of spermatogonia has been described in cryptorchid prepuberal tests by Cooper (4) and Nelson (5). Whether this represents a deleterious effect secondary to maldescent or whether it simply reflects an inherent gonadal congenital imperfection in some way related to the etiology of the cryptorchidism, is not clear at the present time.

The effects of cryptorchidism on the retained adult testis are well known. In general, the intensity of the gross and histologic changes is proportional to the age of the patient and the degree of maldescent. The gland is smaller, softer and less elastic than the scrotal organ. Varying degrees of fibrosis and cellular degeneration and atrophy are encountered.

The mildest alterations are found shortly after puberty. These consist of an inhibition of spermatogenesis to the extent that only few, if any, spermatocytes are present; the parent spermatogonia are reduced in number and show decreased mitotic activity. Sertoli cells are evident in large numbers. The tubules are smaller than normal in diameter and already manifest peritubular fibrosis (5). As the subject grows older, there is progressive shrinkage in tubular size with degeneration of the germinal epithelium, so that eventually small numbers of Sertoli cells constitute the only tubular epithelial remnants. Simultaneously, the tunica propria and peritubular connective tissue become thickened and fibrotic so that ultimately the tubules lose even their Sertoli cells and are transformed into small completely sclerotic structures. Interstitial cells of Leydig usually persist for a considerable number of years.

It is thus evident that the histologic appearance of the very small canalicular testis described in this communication is that of a congenitally defective gonad. No cells resembling spermatogonia could be definitely identified after careful examination of several sections. Sertoli and Leydig

cells were also absent. The small immature tubules were populated exclusively by undifferentiated cells. The microscopic picture cannot be interpreted as a secondary effect of cryptorchidism because of the absence of thickening and fibrosis of the tunica propria and peritubular connective tissue. If cryptorchidism were responsible for the complete absence of spermatogonia, fibrosis would be conspicuous. Furthermore, Sertoli and Leydig cells, absent in this instance, usually persist in the retained testis well into middle age, and their final disappearance also occurs in association with advanced tubular and interstitial tissue sclerosis. In fact, there is very little, if any, histologic evidence of a deleterious effect due to the maldescent *per se*.

Because the patient lapsed from observation it was impossible to obtain additional urine specimens for more precise measurement of the urinary gonadotropin excretion. It is definite that this was not elevated but it cannot be stated whether it was subnormal or within normal limits. A normal urinary gonadotropin titer would be most unusual and is not to be expected in eunuchoidism of the degree exhibited by this individual. It is more than likely that the urinary gonadotropins were low or absent. Under these circumstances the patient would fit into the category described as "idiopathic eunuchoidism-with-low-FSH" (7,8) or "hypogonadotrophic eunuchoidism" (9).

This condition is due to a selective impairment of the gonadotropic function of the adenohypophysis with preservation of its other tropic activities. Its etiology is unknown and may well be congenital. Biopsies are reported to show "unstimulated testes" (9) and absence of germ cell proliferation (8). Spermatogonia are present but Sertoli cell maturation and Leydig cells are usually absent. The histologic appearance is that of a prepuberal testis. That these gonads are morphologically and functionally competent is indicated by their good response to gonadotropin stimulation. Heller and Nelson (10) reported Leydig cell activation and spermatogenesis in these patients after treatment with chorionic gonadotropin combined with purified pituitary follicle-stimulating hormone. Associated cryptorchidism is occasionally encountered in this disorder (7, 11). Howard (12) has the impression that it occurs in 20 to 25 per cent of the cases.

Even if the patient described in the present report represents an instance of idiopathic pituitary gonadotropic underfunction, his testicular histopathologic pattern is apparently entirely independent. Although failure of Sertoli and Leydig cell development may at times be associated with, or due to inadequate gonadotropin stimulation, spermatogonia are present in such testes and the latter are of appreciable, but small size. As previously emphasized, the gonad in the present case was extremely

small and the microscopic appearance was characteristic of a congenitally defective rather than a simple prepuberal type of gonad.

Theoretically, the testicular lesion may have arisen as a result of two different pathogenetic mechanisms, In one instance the histologic picture could be due, in part, to an intrinsic congenital defect (germinal aplasia) and, in part, to deficient gonadotropic stimulation by the adenohypophysis. In favor of this possibility is the previously mentioned fact that absent Sertoli and Leydig cell differentiation is usually encountered in this form of hypopituitarism. Opposed to this possibility is the minute size (0.5 cm.) of the gonad in the present case. Such extremely small testes have not been described in patients with idiopathic hypogonadotropic enunuchoidism. Similarly, the strikingly small size cannot be attributed to germinal aplasia or to cryptorchidism.

On the other hand, the histologic findings and the size of the gland support the alternative hypothesis by which the entire lesion is explained on the basis of a common intrinsic congenital defect. Only by means of adequate gonadotropin therapy is it possible to determine whether the absence of Sertoli and Leydig cells in an adult is congenital or due to defective stimulation. Since this therapy was not employed, it is impossible to decide this point. Nevertheless, the size and structure of the gland suggest that it is most likely totally incompetent and therefore incapable of reacting to normal or to induced stimulation.

It is not known whether a similar lesion exists in the opposite gonad, if the latter is present at all. However, this is of no pathogenetic significance, since a congenital testicular lesion has already been established in this patient. The association of a congenital anomaly in the testis with an idiopathic and probably congenital disorder of the adenohypophysis would not be surprising. Developmental defects are often multiple and may simultaneously involve somatic and gonadal structures.

The lesion exemplifies developmental failure of all three testicular cellular components with the formation only of rudimentary tubules containing primitive undifferentiated cells. It differs from other instances of congenital testicular defect, such as germinal aplasia occurring in otherwise normal men (13–16), or in familial hypogonadism (17). Doubtless other varieties and degrees of testicular dysgenesis exist, since this field is virtually unexplored. Taken as a whole, the various morphologic manifestations of defective testicular genesis are probably responsible for a significant number of testicular lesions loosely called "immature," "hypoplastic" and "atrophic." A few reports in the recent literature (18–20) attest to the growing interest in congenital hypogonadism. Further studies are required for more complete characterization of the individual congenital testicular lesions which may be encountered.

SUMMARY

A 23-year-old male patient with prepuberal eunuchoidism is presented. Histologic examination of an extremely minute canalicular testis disclosed evidence of a profound congenital defect which was responsible for the hypogonadism. This was characterized by the presence of rudimentary tubules containing only undifferentiated cells. Spermatogonia, Sertoli and Leydig cells were absent.

Acknowledgment

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REFERENCES

1. Zondek, B.: Über die Hormone des Hypophysenvorderlappens. IV. Darstellung des Follikelreifungshormons (Prolan A).—Methodik der klinischen Harnanalyse zum Nachweis des Prolan, *Klin. Wchnschr.* **9:** 1207–1209 (June 28) 1930.
2. Klinefelter, H. F., Jr; Albright, F., and Griswold, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3:** 529–544 (Oct.) 1943.
3. Wangensteen, O. H.: The undescended testis. An experimental and clinical study, *Arch. Surg.* **14:** 663–731 (Mar.) 1927.
4. Cooper, Eugenia, R. A.: The histology of the retained testis in the human subject at different ages, and its comparison with the scrotal testis, *J. Anat.* **64:** 5–27 (Oct.) 1929.
5. Nelson, W. O.: Mammalian spermatogenesis: effect of experimental cryptorchidism in the rat and non-descent of the testis in man, *Recent Progr. Hormone Res.* **6:** 29–62, 1951.
6. Sniffen, R. C.: The testis. I. The normal testis, *Arch. Path.*, **50:** 259–284 (Sept.) 1950.
7. Fraser, R. W.; Forbes, A. P.; Albright, F.; Sulkowitch, H., and Reifenstein, E. C., Jr.: Colorimetric assay of 17-ketosteroids in urine: a survey of the use of this test in endocrine investigation, diagnosis, and therapy, *J. Clin. Endocrinol.* **1:** 234–256 (March) 1941.
8. Howard, R. P.; Sniffen, R. C.; Simmons, F. A., and Albright, F.: Testicular deficiency: a clinical and pathologic study, *J. Clin. Endocrinol.* **10:** 121–186 (Feb.) 1950.
9. Heller, C. G., and Nelson, W.O: Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis and treatment, *J. Clin. Endocrinol.* **8:** 345–366 (May) 1948.
10. Heller, C. G., and Nelson, W. O.: The testis-pituitary relationship in man, *Recent Progr. Hormone Res.* **3:** 229–255, 1948.
11. Nelson, W. O.: Personal communication.
12. Howard, R. P.: Personal communication.
13. Engle, E. T.: The testis and hormones, *in* Problems of Ageing, ed. 2, edited by E. V. Cowdry. Baltimore, The Williams & Wilkins Co., 1942, pp. 475–494.
14. Engle, E. T.: The testis biopsy in infertility, *J. Urol.* **57:** 789–798 (May) 1947.

15. TRABUCCO, A.: Esterilidad congenita en el hombre, *Medicina,* Buenos Aires **5:** 369–387 (July) 1945.
16. DEL CASTILLO, E. B.; TRABUCCO, A., and DE LA BALZE, F. A.: Syndrome produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells, *J. Clin. Endocrinol.* **7:** 493–502 (July) 1947.
17. SOHVAL, A. R., and SOFFER, L. J.: Congenital familial testicular deficiency, *Am. J. Med.* (in press).
18. HELLER, C. G.; NELSON, W. O., and ROTH, A. A.: Functional prepuberal castration in males, *J. Clin. Endocrinol.* **3:** 573–588 (Nov.) 1943.
19. RYAN, E. J., and MCCULLAGH, E. P.: Congenital hypogonadism in the male, *Proc. Central Soc. Clin. Research* **19:** 43 (Nov.) 1946.
20. MCCULLAGH, E. P.: Sex hormone deficiencies. Some clinical considerations, *Recent Progr. Hormone Res.* **2:** 295–344, 1948.

A TWO-YEAR FOLLOW-UP ON THE EFFECT OF PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH), CORTISONE AND CORTISONE OINTMENT IN A CASE OF PEMPHIGUS FOLIACEUS*†

CHARLES D. BONNER, M.D. AND F. HOMBURGER, M.D.

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THE 52-year-old white woman described in this paper, in whom the diagnosis of pemphigus foliaceus was conclusively established, has been the subject of investigations reported in two previous communications (1, 2); and more recent photographs of this patient have appeared in Selye's Annual Report on Stress, 1951 (pp. 425–428).

Observations previously reported demonstrated that remissions could be induced repeatedly with ACTH and that relapses invariably followed the withdrawal of this hormone; further, that after becoming completely refractory to ACTH this patient still responded to cortisone given systemically, and that remissions thus obtained could be enhanced by topical applications of cortisone to persistent lesions. In addition, observations made since October 1950 have shown that by various combinations of ACTH and cortisone it has been possible to control a "terminal" case of generalized pemphigus foliaceus to the point where, at present, the patient has returned as a useful member to her community.

The administration of ACTH restored the patient's lost responsiveness to cortisone. Conversely, the use of cortisone restored responsiveness to ACTH at times when exacerbations of the disease occurred while the patient was receiving ACTH. It was later found that ten-day courses of 100 mg. of ACTH per day given every two to three months simultaneously with continued cortisone therapy, maintained or restored this patient's responsiveness to cortisone.

This report covers observations made after the twenty-sixth week of treatment (1). At that time the patient was well controlled by ACTH and

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* This study was aided in part by grants from the Damon Runyon Memorial Fund for Cancer Research, The American Cancer Society, and The U. S. Public Health Service, National Cancer Institute, Bethesda, Maryland.

† Work completed under a Damon Runyon Clinical Research Fellowship recommended by the Committee on Growth of the National Research Council and administered by The American Cancer Society.

the disease continued in partial remission for fifteen weeks. During the forty-third week of our study (seventeenth week of present study) new lesions appeared, the circulating eosinophils rose to 800 per cubic millimeter, and the 17-ketosteroid excretion fell to zero.

Cortisone administered in doses of 100 mg./day failed to influence the skin lesions, but produced excellent results when given at dosage levels of 200 mg./day for ten days. Thereafter 100 mg./day was an adequate

FIG. 1. (Left) appearance of the patient before treatment, in the first weeks of observation. (Right) in the 105th week of treatment.

maintenance dose. Repeated attempts to maintain improvement on a dosage scale below 100 mg./day met with failure. Lesions of the face, legs and beneath the breasts persisted until cortisone ointment was applied; this additional therapy produced definite beneficial effects. During the period of combined systemic and topical cortisone treatment the eosinophil count dropped to 88 per cubic millimeter.

During the seventy-fourth week new lesions developed on the abdomen, chest, back, breasts, arms and legs, associated with a rise in the eosinophil count. The use of ACTH was instituted again, with clearing of the lesions; the eosinophil count and sedimentation rate returned to normal levels.

A maximum improvement was obtained during this therapeutic period, accompanied by low sedimentation rates and eosinophil counts.

At the eighty-eighth week in this patient's course she was again given cortisone because of the expense involved in prolonged ACTH therapy. Her improvement was maintained and there was a gradual bleaching of the pigmented areas on her lower legs.

By the ninetieth week her disease was controlled and she was discharged

FIG. 2. (Left) close-up of the patient's face in the period before therapy. (Right) in the 105th week of treatment.

home on continued cortisone therapy, to return for periodic follow-up examinations.

During the ninety-fifth week, three isolated lesions developed on the arm, shoulder and breast. In addition to the maintained cortisone therapy a course of ACTH (100 mg. for ten days) was given and the new lesions disappeared, save for one on the left breast. Throughout this period of combined therapy the eosinophil count and sedimentation rate remained low. With the administration of potassium chloride (3 Gm. per day) it was possible to maintain a constant normal serum potassium level. For the twenty-seventh and twenty-eighth weeks Lipo-adrenal Extract was given intramuscularly without apparent success.

At the time of this report and after two years of both adrenal stimulation and substitution therapy, the patient has a normal blood pressure, mild hirsutism, mild Cushing-syndrome facies, and occasional transient glycosuria. She is leading a normal life, which seemed a remote probability at the time of her admission to the hospital, when she appeared as shown in Figures 1 and 2 (left). By contrast, her present status is shown in Figures 1 and 2 (right). Her clinical course from the twenty-sixth through the hundred and fifth week is shown in Figure 3.

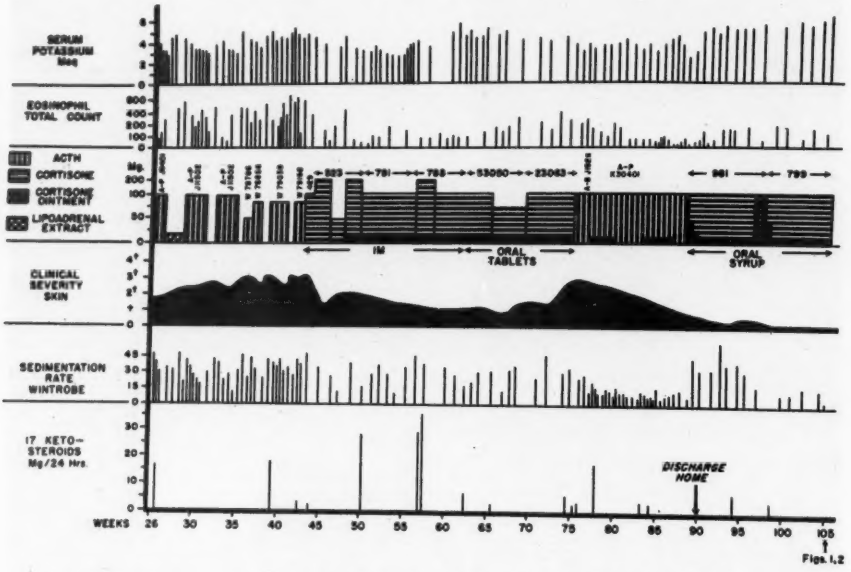

FIG. 3. Clinical course and some of the laboratory studies, from the 26th through the 105th week of observation. The serum potassium is expressed in milliequivalents per 100 milliliters of serum, and the eosinophil counts in numbers per cubic millimeter. The severity of skin lesions was evaluated from Kodachrome photographs and graded arbitrarily from 1+ to 4+ in severity. Sedimentation rate of erythrocytes is expressed in millimeters per hour.

DISCUSSION

The association in pemphigus foliaceus between recurrence of skin lesions and evidence of lowered adrenal function, and the responsiveness of skin lesions to ACTH or cortisone, have been consistently established in this patient over a two-year period. Several patterns of response were observed. Cessation of either ACTH or cortisone, when given systemically, was followed by recurrence of visible skin lesions, as was any attempt to lower the dosage of either drug below 100 mg. per day. After several months on either therapy exclusively, refractoriness occurred and new lesions

appeared. However, at that point, a renewed response could be obtained with the alternate drug which had become previously ineffective. With this varied alternating systemic therapy of cortisone and ACTH, lesions on the face, lower legs and under the breasts still failed to resolve completely until a 12.5 per cent cortisone ointment was applied directly to these lesions in addition to systemic therapy. More undesirable signs appeared with ACTH therapy than with cortisone, and a typical Cushing-syndrome facies, obesity, fluid retention, hirsutism, and glycosuria occurred intermittently while ACTH was given. The blood pressure was never elevated. With every course of ACTH a strange taste sensation was experienced by the patient. During the last course of ACTH there were additional side reactions never before experienced, such as faintness, hot flashes, weakness and general malaise. It is uncertain to what degree this can be attributed to impurities of a particular lot of ACTH. Cortisone therapy resulted in fewer side effects. The patient's weight during cortisone therapy fluctuated about 10 pounds, which was considerably less in amount than during ACTH therapy.

It would seem at this time that the optimal treatment for this patient is oral cortisone (100 mg./day), plus cortisone ointment applied topically to small persistent lesions, supplemented by ten-day courses of ACTH (100 mg./day) at two- or three-month intervals. In this manner it may be possible to maintain the remission on cortisone therapy for a long period of time. Normal serum potassium levels are maintained by 3 Gm. of potassium chloride given orally each day.

SUMMARY

In a 52-year-old white female, admitted two years previously in the terminal stages of pemphigus foliaceus, a remission of the disease has been maintained by the use of ACTH and cortisone alternately, or in combination. Administration was by the intramuscular route in the case of ACTH, and orally or by topical application in the case of cortisone. This therapy has allowed the patient to carry on a normal life at home.

Acknowledgments

The cortisone used in this study was obtained through the courtesy of Drs. G. Gibson and Elmer Alpert of Merck & Co., Rahway, N. J. The ointment and creams were either in petrolatum base or in a vanishing cream base. The latter has been found more satisfactory in this particular case.

The ACTH was purchased in part by funds from the U. S. Public Health Service, National Cancer Institute; and in part was obtained through the courtesy of Dr. A. H. Holland, Medical Director of the Armour Laboratories, Chicago, Ill.

The Lipo-adrenal Extract was provided through the courtesy of the Upjohn Company, Kalamazoo, Michigan.

REFERENCES

1. HOMBURGER, F.; BONNER, C. D., and FISHMAN, W. H.: Effect of pituitary adrenocorticotropic hormone (ACTH) in a case of pemphigus foliaceus, *J. Clin. Endocrinol.* **10:** 1591–1601 (Dec.) 1950.
2. HOMBURGER, F., and BONNER, C. D.: Topical cortisone in pemphigus, *Bull. New England Med. Center* **13:** 94–95 (April) 1951.

Letters to the Editor

A RAPID PROCEDURE FOR THE DETERMINATION OF SERUM CHOLESTEROL*

To the Editor:

A simple direct procedure for the determination of total cholesterol in serum has been developed to meet the needs of a large-scale study of endocrinologic changes and lipid metabolism in old age. The procedure requires only the addition of reagents to serum and the measurement of the intensity of the resultant color. The determination is complete in less than thirty minutes.

Sera were analyzed by this rapid procedure in our laboratory and by the classic Schoenheimer-Sperry method in another laboratory.[1] An average deviation of ± 3.5 per cent was found between the results obtained by the two methods in the two institutions.

By the new method, normal values for blood cholesterol of 170 to 240 mg. per 100 ml. of serum have been found. In patients suffering from hypothyroidism, arteriosclerosis, the nephrotic stage of chronic glomerular nephritis, or biliary obstruction, blood cholesterol concentrations from 297 to 1,193 mg. per 100 ml. have been obtained.

PROCEDURE

A. *Preparation of serum unknown.* To 0.1 ml. of serum add in the following order, 0.1 ml. of glacial acetic acid (C. P. reagents are used throughout), 0.5 ml. of *p*-toluenesulfonic acid solution (12 Gm. of *p*-toluenesulfonic acid per 100 ml. of glacial acetic acid), and 1.5 ml. of acetic anhydride; do not stir. Let the mixture stand at room temperature until it cools, and then add 0.2 ml. of concentrated sulfuric acid. Shake vigorously immediately after the acid is added until the precipitate is completely dissolved. After the solution has stood at room temperature for twenty minutes, measure the optical density at 550 mμ.

B. *Preparation of cholesterol standard.* To 0.1 ml. of a glacial acetic acid solution of cholesterol (200 mg. per 100 ml.) add 0.1 ml. of water; then proceed as in step A, omitting the addition of 0.1 ml. of glacial acetic acid.

C. *Adjustment of zero point of the spectrophotometer.* The solution with which the zero point of the spectrophotometer is set before measuring the optical densities of the standards and sera, is prepared by substituting 0.1 ml. of water for serum in step A.

* Presented by title at the Annual Meeting of the Endocrine Society, Chicago, Illinois, June 5–7, 1952.

[1] We wish to thank Dr. L. Lewis Abell of Columbia University for the analyses by the Schoenheimer-Sperry method.

D. *Preparation and reading of blanks.* "Blanks" to correct for interfering substances in serum that absorb at 550 mμ are prepared by the addition of 2.3 ml. of *p*-toluenesulfonic acid solution to 0.1 ml. of serum. Set the zero point of the spectrophotometer with the *p*-toluenesulfonic acid solution before reading the blank.

Calculations

$$\text{mg. cholesterol/100 ml. serum} = \frac{100(a-b)d}{c}$$

a—optical density of serum
b—optical density of blank
c—optical density of standard
d—mg. cholesterol/ml. standard solution

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SALICYLATES, ANTERIOR PITUITARY, ADRENALS AND EOSINOPHILS

TO THE EDITOR:

Referring to the publication of Dr. H. F. Hailman (ACTH and cortisone *vs.* salicylate, *J. Clin. Endocrinol. & Metab.* **12**: 454, 1952), I should like to extend the bibliography dealing with the interrelation between the anterior pituitary or ACTH, the adrenals or cortisone, and sodium salicylate. In April, 1950 (1, 2) we stressed the similarity between the metabolic effects of sodium salicylate and those of ACTH, proposing the hypothesis that the former may act through the intermediary of the hypophyseal-adrenal system. In June of the same year, we presented to the Argentine Society of Biology (3, 4) papers dealing with the effect of sodium salicylate on the ascorbic acid content of the adrenal and the hypophysis of the rat. We observed that 40 mg. per 100 Gm. of body weight, of sodium salicylate administered subcutaneously, caused a fall in the adrenal ascorbic acid content which reached a maximum (−46 per cent) within two hours, maintained this level up to six hours and was still at a low level (−25 per cent) twenty-four hours later; this curve is in contrast with that obtained with adrenalin, in which the effect lasts less than twelve hours. The pituitary ascorbic acid content was increased two and six hours after

the injection, but was slightly below the normal level twenty-four hours later. In the same paper we laid emphasis on the possibility that sodium salicylate would cause eosinopenia and that the latter might help in determining the dosage and controlling the treatment of rheumatic fever with sodium salicylate.

Clinically, we observed (5) that the administration of 3 Gm. of sodium salicylate by mouth in one or in two doses brought about a fall of as much as 90 per cent of the circulating eosinophils, six hours after its administra-

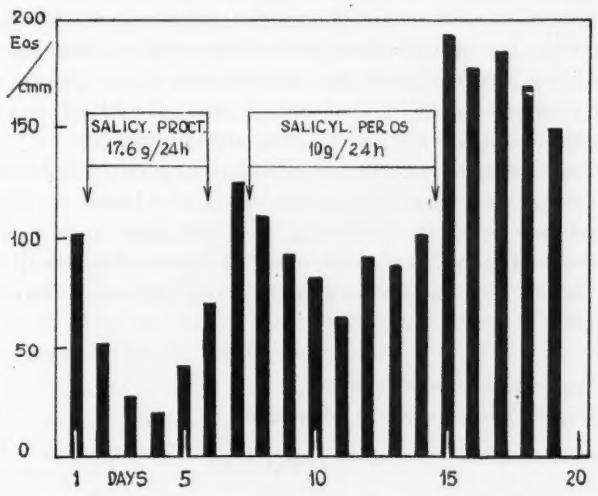

FIG. 1. Number of circulating eosinophils per cubic millimeter, in a case of rheumatic fever treated with sodium salicylate. Daily eosinophil counts are plotted. During the first part of the treatment, sodium salicylate was administered by proctoclysis and during the second part, by mouth.

tion to a normal subject. Performing eosinophil counts two, four and six hours after the administration of sodium salicylate, we observed that the drop in eosinophils was general inversely proportional to the salicylemia values. We therefore concluded that a periodic count of the number of circulating eosinophils constitutes an excellent criterion for the control of the salicylate treatment. A typical example of the behavior of the circulating eosinophils in the course of the treatment of rheumatic fever is illustrated in Figure 1 (6), in which it can be seen that the administration according to our technique (7) of an intensive dosage of salicylates by proctoclysis, together with ammonium chloride, brings about a marked and progressive drop in circulating eosinophils, which reaches a maximum on the fourth day of treatment. Upon discontinuing this treatment the

eosinophils increase in number, attaining a level higher than the initial one; but upon re-initiating the treatment by mouth, the eosinophils again show a decrease. When the treatment is definitely stopped, the eosinophils normally rise until they about duplicate the original value. On the basis of a large number of similar observations, we think that the determination of eosinophil counts every twenty-four or forty-eight hours in the initial stages of the treatment of rheumatic fever with sodium salicylate, constitutes a simple method and possibly a more accurate one than the determination of the degree of salicylemia. If, as we are inclined to think, sodium salicylate exerts its effect through the intermediary of the hypophyseal-adrenal system, the appreciation of the degree of eosinopenia constitutes a more accurate expression of the effectiveness of the dosage used, since it not only depends on the level of salicylates in the blood, but also on the hypophyseal-adrenal reactivity to these concentrations.

The therapeutic effect of sodium salicylate in rheumatic fever cannot be based solely on the dosage used; it should also be based on the hypophyseal-adrenal reaction (8). Following this principle and using the high dosage we recommend, we think that the favorable results which we generally obtain (1) are comparable to those obtained with ACTH and cortisone (8).

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REFERENCES

1. PASQUALINI, R. Q.: El tratamiento de la fiebre reumática; una larga experiencia y una hipotesis personal, *Prensa méd. argent.* **37:** 757, 1950.
2. PASQUALINI, R. Q.: Suprarrenal y reumatismo, *Día médico* **22:** 2108, 1950.
3. PASQUALINI, R. Q.: Corticotrofina y salicilato de sodio en el tratamiento de la fiebre reumática, *Rev. Asoc. méd. argent.* **64:** 332, 1950.
4. PASQUALINI, R. Q.; PASQUALINI, C. DOSNE DE, and GARBERI, J. C.: Acción del salicilato de sodio sobre el sistema hipófiso-suprarrenal, *Rev. Soc. argent. de biol.* **26:** 120, 1950.
5. PASQUALINI, R. Q.; PASQUALINI, C. DOSNE DE, and GARBERI, J. C.: Action du salicylate de soude sur le système hypophyso-surrénal, *Compt. rend. Soc. de biol.*, Paris, **145:** 122, 1951.
6. PASQUALINI, R. Q.: Eosinopenia salicilica, *Prensa méd. argent.* **39:** 490, 1952.
7. PASQUALINI, R. Q., and LASCALEA, M. C.: Tratamiento de la fiebre reumática con salicilato de sodio y cloruro de amonio, *Prensa méd. argent.* **35:** 1091, 1948.
8. PASQUALINI, R. Q.: Stress; Enfermedades de Adaptación, ACTH y Cortisona. El Ateneo, Buenos Aires, 1952.

CORTISONE *VS.* SALICYLATE

To the Editor:

The recent article by Dr. Hailman (*J. Clin. Endocrinol. & Metab.* **12**: 454 (April) 1952) dealing with cortisone versus salicylate, has prompted me to point out the following:

In 1950 we reported work on salicylates, stress and cortisone (1) (2). The amount of "antiarthritic adrenal hormones" mobilized by our salicylates doses failed, however, to reach or approximate the amount of cortisone-hydrocortisone available for the organism in producing a cortisone remission of rheumatoid arthritis. The daily excretion of urinary cortisone-active material, determined by Cope's biologic method (3), was unaltered in our further experiments. Perhaps the cortisone-hydrocortisone utilization of the tissue level increases in such cases. The explanation of the therapeutic effect of salicylate injury in rheumatic fever remains unsolved. Epinephrine and the cortical hormones mobilized via adrenocorticotropin play only a part in it. There are also factors which affect the suprahypophyseal neural structures, and others which act at the cellular level. Salicylate, in some manner, seems to act in the presence of, but not through the adrenals.

E. Kelemen, M.D.

I. Belklinika,
Szeged, Hungary

REFERENCES

1. Kelemen, E.; Majoros, M., and Tanos, B.: Salicylates in acute osteomyelitis, *Lancet* **2**: 457, 1950.
2. Kelemen, E.; Majoros, M.; Iványi, J., and Kovács, K.: Salicylates, stress and cortisone, *Experientia* **6**: 435, 1950.
3. Cope, C. L.: Detecting cortisone in body fluids, *Brit. M. J.* **1**: 271, 1951.

The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send FOUR COPIES of the title and abstract to the Vice-President, Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore 5, Maryland, not later than FEBRUARY 1, 1953. **It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:**

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three additional copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953*.

FIFTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

Sponsored by

THE ENDOCRINE SOCIETY

Roney Plaza Hotel

Miami Beach, Florida *November 3–8, 1952*

The faculty will consist of 20 clinicians and investigators eminently qualified in the fields of endocrinology and metabolism.

The program will consist of lectures, clinics, laboratory demonstrations and scientific exhibits.

The 64 lectures to be presented will cover all phases of endocrinology and metabolic disorders. Ample time will be given for discussions and answers at the end of each session and registrants are encouraged to contact members of the faculty for individual discussions. The entire program has been designed to interest not only the specialist but those in general practice and has been approved by the American Academy of General Practice for postgraduate credit.

A simultaneous translation of all lectures into Spanish will be provided through earphones.

A copy of the proceedings of the Assembly will be available to all registrants.

The Roney Plaza with its tropical gardens, outdoor swimming pool, putting greens, private beach and Cabana Club, has long been recognized as one of Florida's great beach hotels and offers special convention rates to members of this Assembly. The Surf Room, in which the sessions will be held, is delightfully air conditioned and has subdued lighting which permits taking of notes and viewing lantern slides with comfort.

A fee of $75 will be charged for the entire course and the attendance will be limited to 200. *Registration will be in the order of checks received and will close on October 3, 1952.* Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Please forward application on your letterhead, together with check payable to The Endocrine Society, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 N. Walker Street, Oklahoma City 3, before October 3, 1952. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida, and the hotel advised that you are attending this Postgraduate Assembly.

[For program, *see* August issue of the Journal.]

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9, 1953*, in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953*. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.

DR. KONRAD DOBRINER

KONRAD DOBRINER, M.D.

(October 14, 1902–March 10, 1952)

Konrad Dobriner was born on October 14, 1902 in Elberfeld on the Rhine, Germany. He was the son of Dr. Paul Dobriner, a prominent industrial chemist of the I. G. Farben-industrie who developed many organic analytic processes and transmitted an early interest in chemistry to his son. Konrad's youth was spent in Leverkusen, and he was graduated from the Realgymnasium of Lennep in 1922. He took his pre-clinical training at the University of Freiburg in Breisgau and, as was customary, continued his medical training at another school, the University of Munich, from which he was graduated in 1927.

After completing his internship at the Krankenhaus München-Schwabing in 1928, Dr. Dobriner was admitted to the practice of medicine. He served as physician at the Krankenhaus for the next five years during which he completed his training as a specialist in internal medicine. In 1933, he was awarded the Doctorate of Medical Science, Magna Cum Laude, from the University of Munich.

Dr. Dobriner's lifelong studies of the metabolic processes in human disease were begun early in his career when he worked with Otto Neubauer. His doctoral dissertation was concerned with alimentary fructosemia in patients with liver disease. During these days in Munich, his interest in porphyrins was stimulated by frequent contacts with Hans Fischer. On his arrival in this country in 1934, Konrad Dobriner began his now classic studies in porphyrin metabolism. These investigations, initiated at the University of Rochester, were brought to completion at the Rockefeller Institute for Medical Research.

A second major phase in his investigative career began when Konrad Dobriner moved to the Memorial Hospital in 1939. As part of the new research policy of the Hospital, a study of the metabolism of steroid hormones was instituted. With his customary zeal and enthusiasm, Konrad Dobriner quickly expanded the project into a major investigation of the role of the steroid hormones in health, in cancer, and in other diseases. Despite the war and its attendant dislocations and difficulties for scientific studies, this broad program advanced so steadily that when the Sloan-Kettering Institute was organized his research formed a major portion of the program of the new laboratories.

The scientific work of Konrad Dobriner was carried on in an atmosphere of strong personal attachments to his fellow workers in the field. His approach was never that of a scientific recluse. At scientific meetings, in personal discussion and by an audacious use of his adopted language, he con-

stantly sought to present his convictions and those of his colleagues. His success in these efforts won him the sincere affection even of those associates with whom he was in scientific disagreement. With undeviating logic and unshakable tenacity he combined those qualities of warmth and human interest which elicited appreciation of his ideas and his enthusiasms. Few will forget his remarks and comments, and the wealth of his scientific contributions remains as a landmark in the field to which he devoted his life.

MEMORIAL TO KONRAD DOBRINER

And so today we hold the bright spirit of our comrade close and in the focus that our sorrow gives see him as clearly as we can and mark his ways. And if we ask from the "Leaves of Grass,"

> "Oh what shall we hang on the chamber walls?
> And what shall the pictures be that we hang on the walls
> To adorn the burial house of him we love?"

we recall the sage of this island who knew these streets and rivers and an earlier generation of the crowds about us, a poet indeed of hospitals and of the sick and wounded there. For this voice remains the voice of our country's better self, of all-absorbing love of life, and of welcome to all gifts, great and small, to long thoughts and to warm endeavor from all mankind. Our comrade knew this welcome. He was at home with us. He was never an alien here. And at one with this spirit his heart was open to all and his mind to all endeavor. For such was the hospitality of his soul.

In the heroic enterprise which seeks to take from every kind of knowledge the proper tools to break the grip of cancer and to employ every manner of man, every skill and temperament that can use these tools and can judge the use made, our comrade found his own true place. For here no barrier should stand between one man and his fellow, between one particular line of work and another. And so unflagging he went from man to man, in sympathy with each, seeking the best that each could give and giving in return. He explored many techniques and mastered those most needed. But within and beneath the most intricate detail of all his skillful chemistry, one always felt the restless, striving purpose of his work. To give this purpose a sharpened edge, he theorized boldly on the nature of the neoplastic process and applied his own best skill toward direct solution. For the mind must try its strength against the major hazard of the course, lest there be no forward movement to a great design. And our comrade understood, with his chief and colleague and friend of many years, the Director of this Institute, that it is the function of leadership to assume these risks that others will not venture. And so they shared the unquiet hours of great responsibility. From this has come the substance of important work for all of us, and for you of the Institute a transfiguration into life of the motto on your wall* and a tradition for your keeping.

Pictures of the movement of large events and of our comrade's place in them belong here truly, and it is some comfort to see the greater scale. But each of us is small and in some sense alone and each would hang a picture that only he could draw of where our comrade touched him. Some fragment from a night's long talk, some raillery against pretence, some clever sally that reduced "grand affairs" to their basic human terms, some useful frankness, some impatience with inaction that shook matters down, some merry welcome, some artless kindness, or his own sweet confidence to support our need. Something then from each of us from the warmth of long camaraderie, for the secret places of the heart are cold enough without such fires as these.

So one may tell his children in the time to come that there were many here and they came because they loved him. For it is ordained that this will give them pride and joy and strength and ease their pain.

ALLAN T. KENYON, M.D.

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* "Within these walls a few labor unceasingly that many may live."